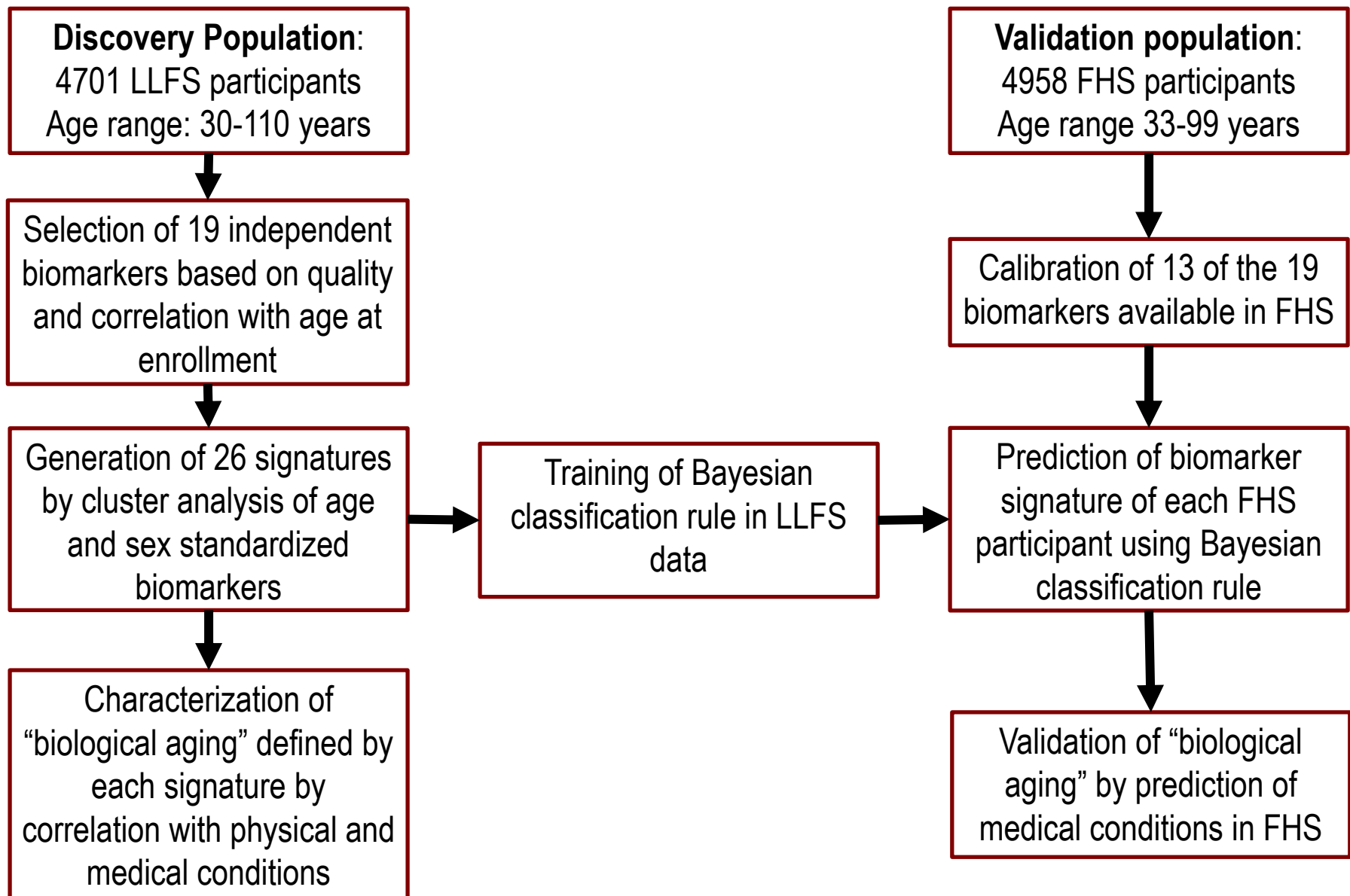


Paola Sebastiani, Bharat Thyagarajan, Fangui Sun, Nicole Schupf, Anne B Newman, Monty Montano, Thomas T Perls

BIOMARKER SIGNATURES OF AGING: SUPPLEMENT FIGURES 1-22

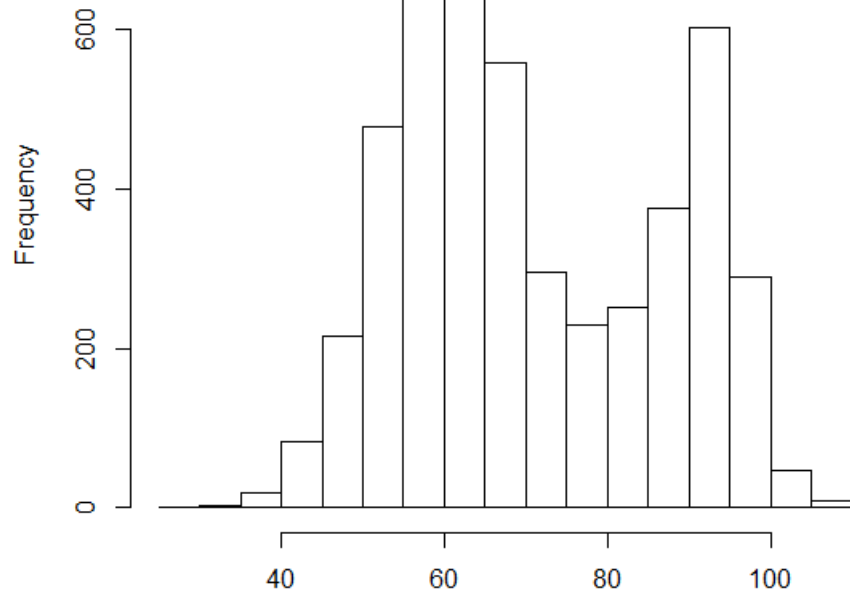
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- ✱ Supplement Figure S2: Distribution of age at enrollment in LLFS
- ✱ Supplement Figure S3: Overview of cluster analysis to discover biomarker signatures
- ✱ Supplement figure S4(a-b): age and sex distributions of biomarkers
- ✱ Supplement Figures S5 through S17: description of 26 biomarker signatures in LLFS
- ✱ Supplement Figure S18a through S18p: Age and sex specific distribution of 19 biomarkers in clusters 1-17. Red and blue denote females and males in cluster 1; magenta and cyan denote females and males in the cluster described in the title page
- ✱ Supplement Figure S19: example of lab-bias in the measurement of albumin
- ✱ Supplement Figure S20a through S20w: distribution of externally standardized biomarkers in FHS data using LLFS means and standard deviations
- ✱ Supplement Figure S21: Distribution of biomarker signatures in LLFS and FHS offspring
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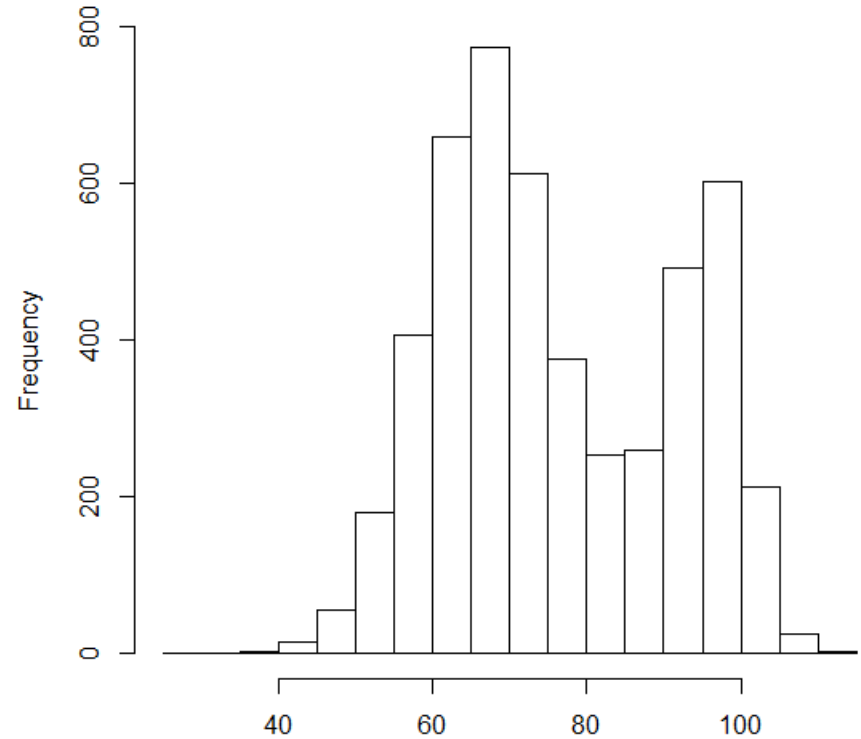


Supplement Figure S1: Flow chart of the analytic approach

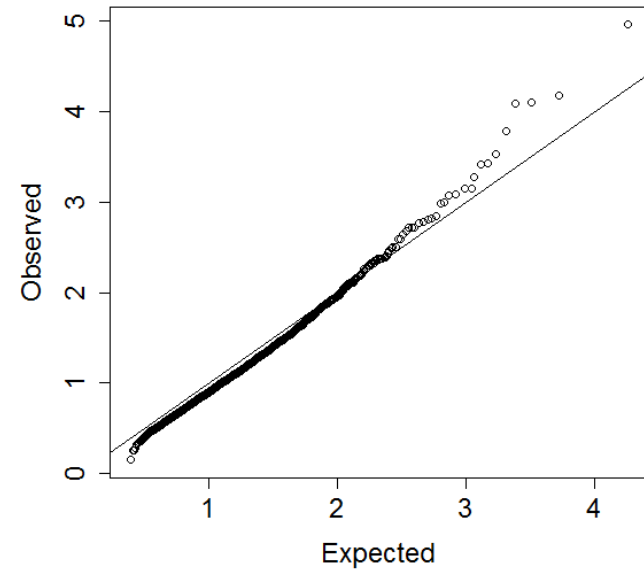
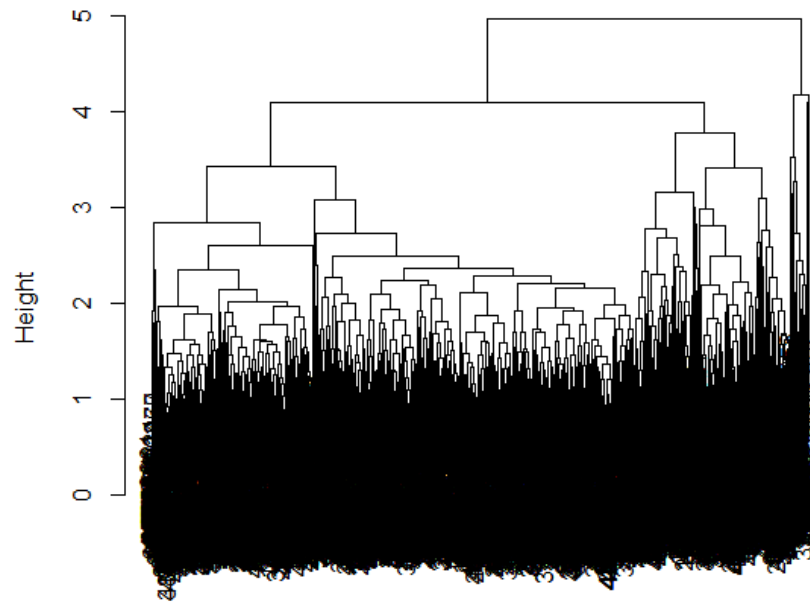
Age at enrollment



Age at last contact

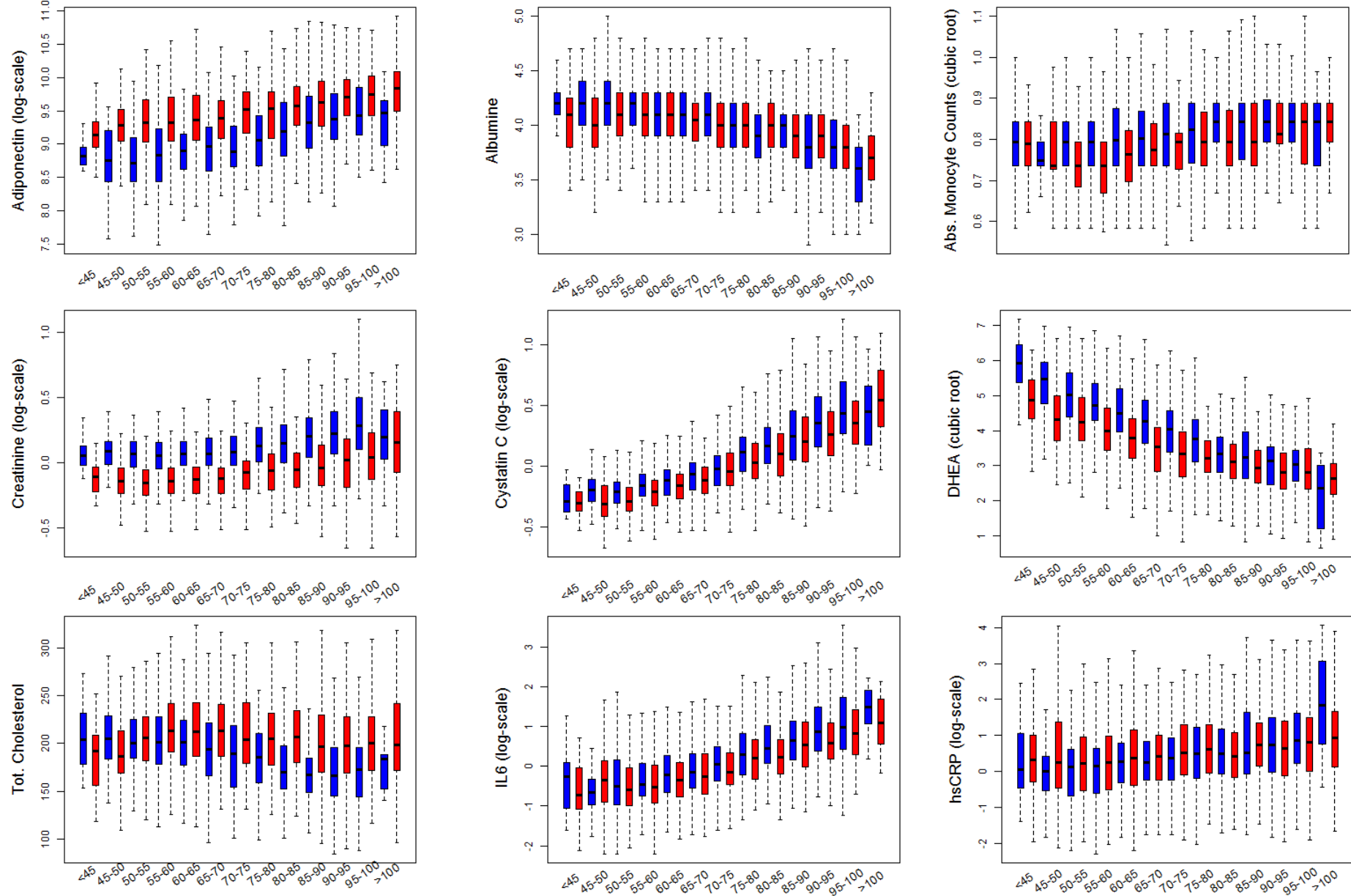


Supplement Figure S2: Distribution of age at enrollment and age at last contact in the 4701 LLFS participants included in the analysis

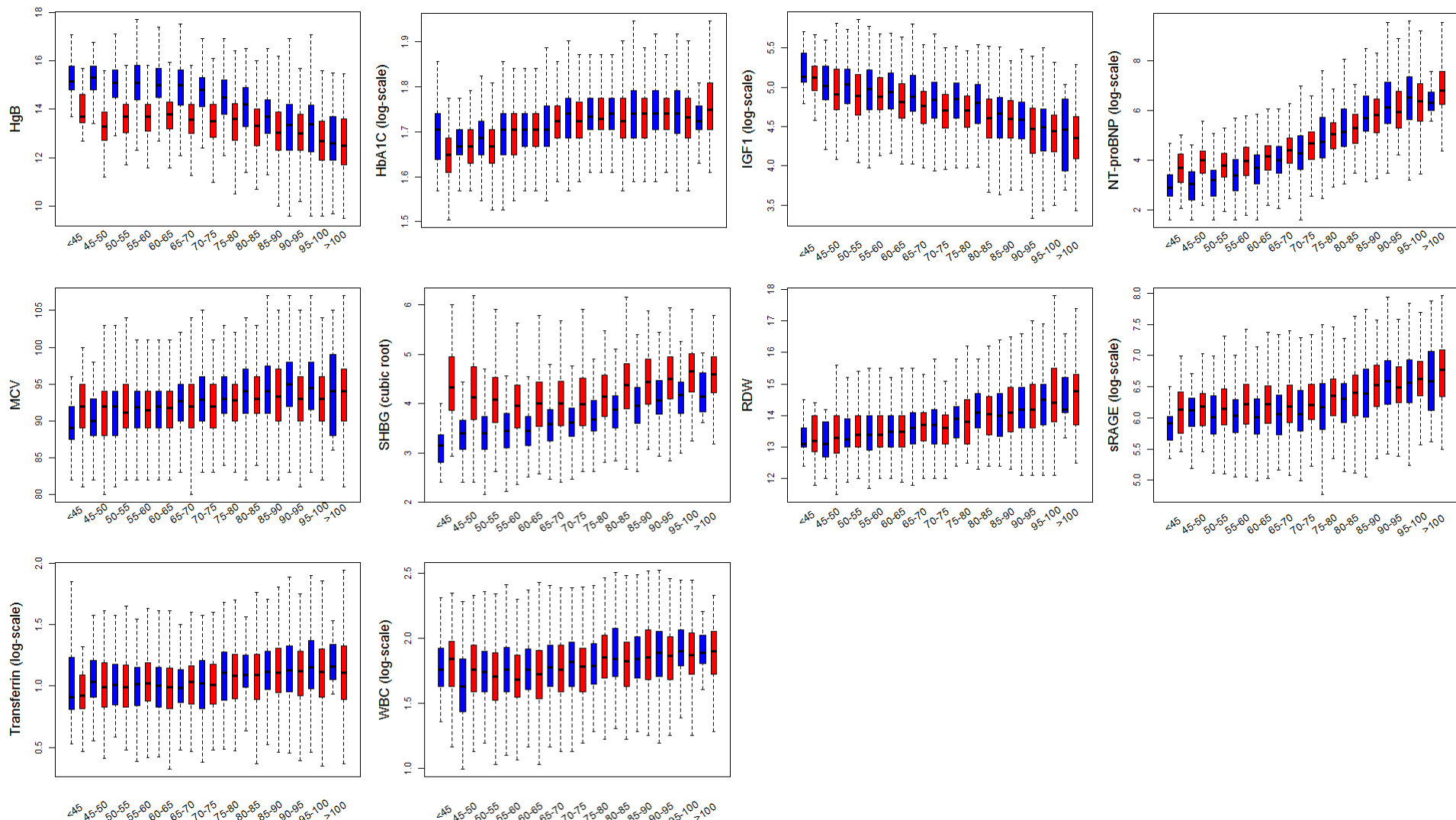


α	Cluster Size for Different Levels of Significance																								N clusters														
0.001	2298					1160				401					462						251				102			18			3	5	4	10					
0.002	2293					5	1160				401					414						48			211				40		102			12	6	3	5	4	14
0.003	2293					5	1128			32	387			11	3	414						28	14	6	211				40		102			12	6	3	5	4	19
0.004	2262			31	5	1128			32	387			11	3	178		140		96	28	14	6	178				33	29	11	91		11	8	4	6	3	5	4	26
0.005	2262			31	5	1120		8	32	387			11	3	159	19	140		96	28	14	6	178				33	29	11	91		11	8	4	6	3	5	4	28
0.006	1919	343	31	5	1120		8	32	195	192	3	8	3	159	19	131	9	96	28	14	6	178				33	29	11	61	30	11	8	4	6	3	5	4	33	

Supplement Figure S3: *Top left* – dendrogram displaying the arrangement of subject profiles by hierarchical clustering with complete linkage. The distances are normalized by the number of biomarkers ($n=19$). *Top right:* QQ-plot displaying the observed and expected distances used in hierarchical clustering (height of branch nodes). Departure from the diagonal line suggests that there are significant clusters in the data. *Bottom:* Cluster composition for different levels of significance α (first column). Colors track clusters that are robust with respect to different levels of significant. The number of clusters ranges from 10 for $\alpha=0.1\%$ to 33 $\alpha=0.6\%$ but most of the differences are in the generation of new clusters with very small number of individuals. With $\alpha=0.4\%$ the algorithm detects 26 clusters, and the most noticeable difference from the clusters detected with α between 0.1% and 0.3% is the split of the cluster with 462 participants into 3 smaller clusters of 96, 140 and 178 participants (Clusters highlighted in blue). For all subsequent analyses, we used the 26 clusters detected for a significance level of 0.4% that provides a good compromise between number of clusters and error rate.



Supplement Figure S4a: Age and sex distribution of selected biomarkers (blue=males, red=females.)



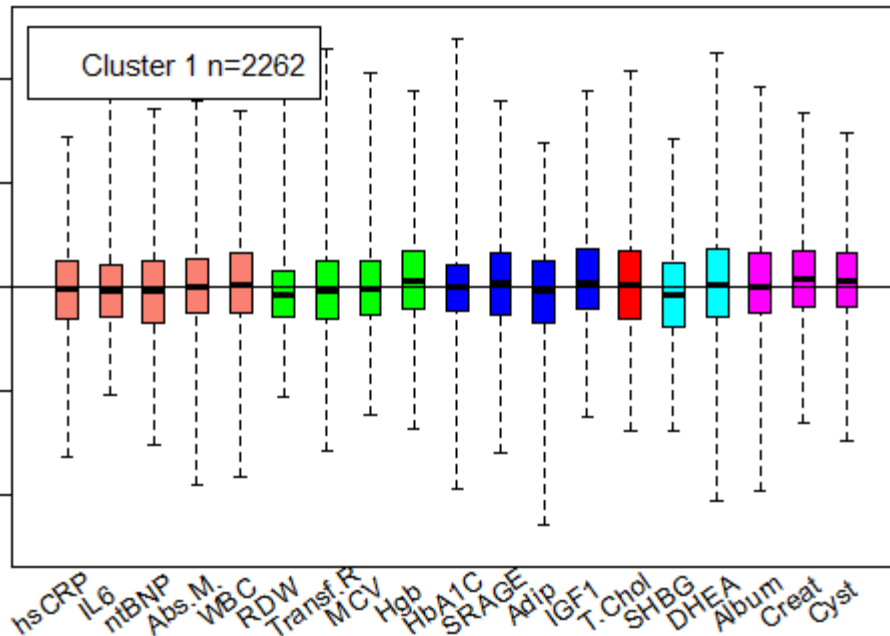
Supplement Figure S4b: Age and sex distribution of selected biomarkers (blue=male, red=females.). Mean and standard deviation per age group were used to define the age and sex specific z-score. Outliers were removed using 2.5% trimmed means.

Supplement Figure S5: Profiles 1 and 2

“Referent profile” : biomarkers are equal to the value expected for age/sex

Cluster:

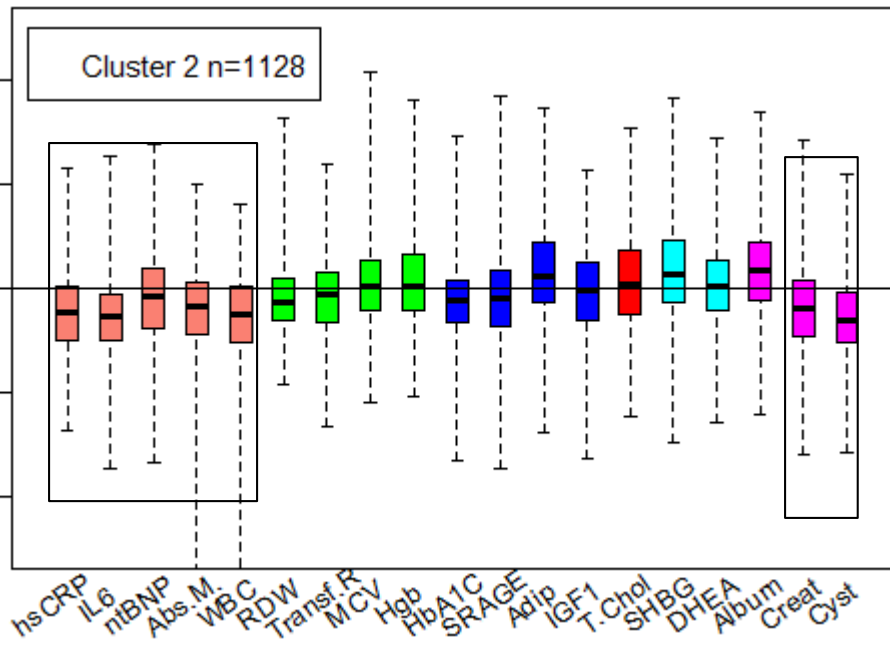
37% born < 1935; median age 90
63% born \geq 1935; median age 60
54% females



Profile 2: a group of biomarkers lower than the value expected for age/sex

Cluster:

41% born < 1935; median age 90
59% born \geq 1935; median age 61
55% females

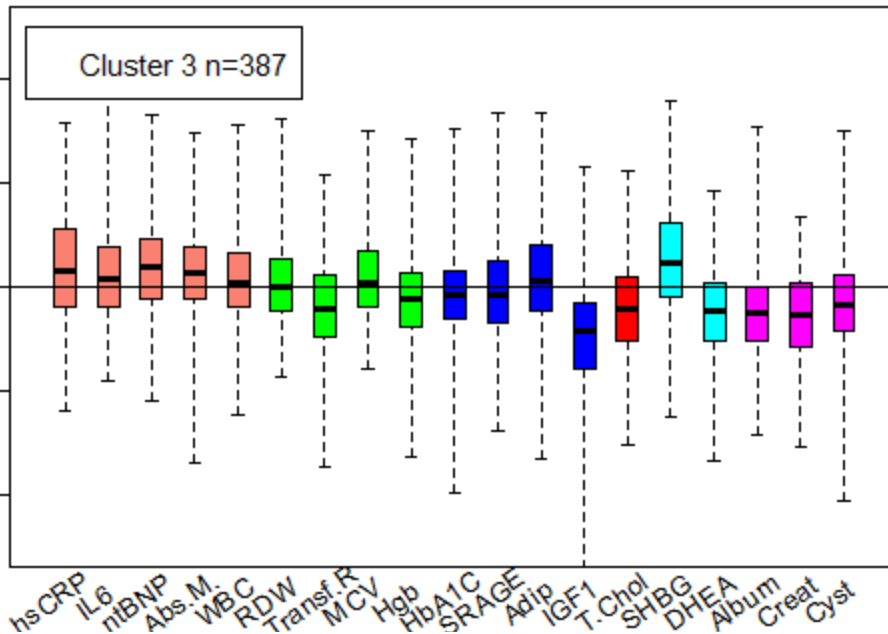


Supplement Figure S6: Profiles 3 and 4

Profile 3: higher than average inflammation, low DHEA and albumin suggesting frailty

Cluster:

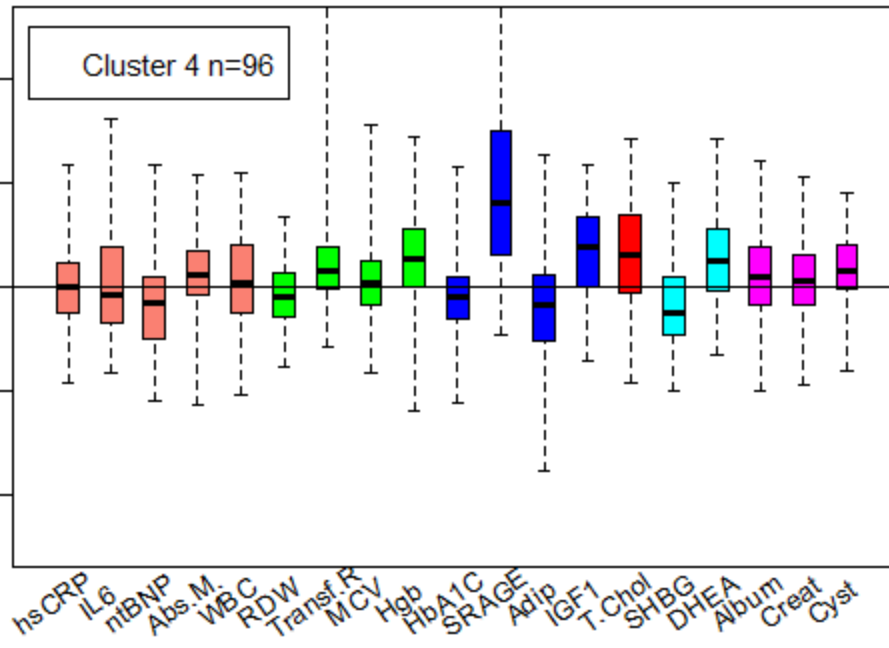
34% born < 1935; median age 91
66% born \geq 1935; median age 60
59% females



Profile 4: extremely elevated sRAGE, IGF1 and cholesterol

Cluster:

36% born < 1935; median age 92
64% born \geq 1935; median age 61
55% females



Supplement Figure S7: Profiles 5 and 6

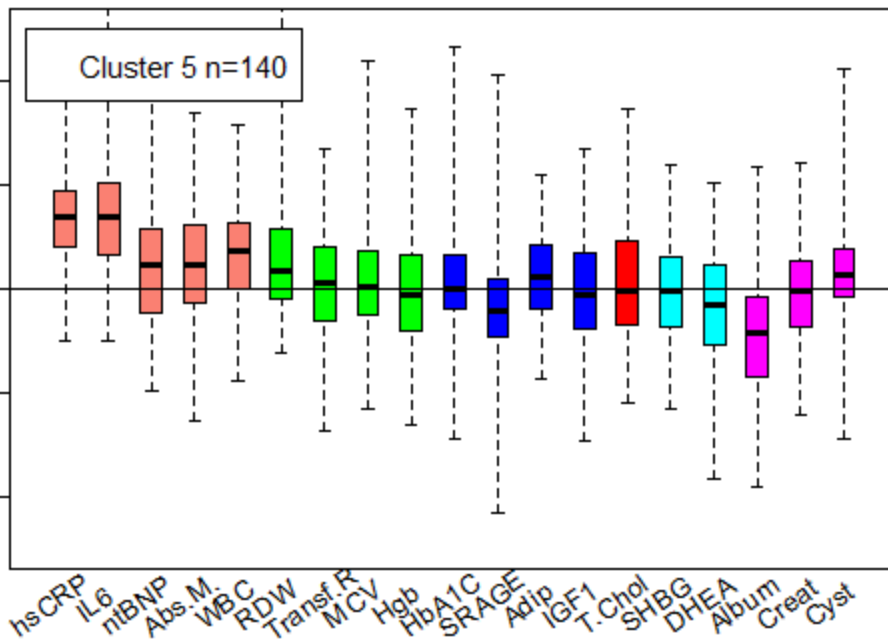
Profile 5:

Cluster:

41% born < 1935; median age 91

59% born \geq 1935; median age 59

46% females



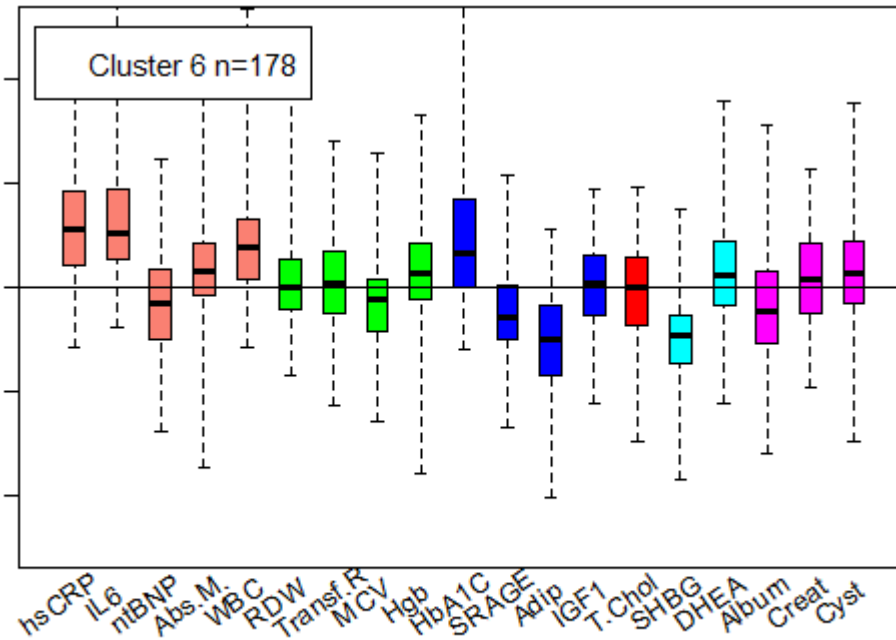
Profile 6:

Cluster:

36% born < 1935; median age 90

64% born \geq 1935; median age 62

62% females



Supplement Figure S8: Profiles 7 and 8

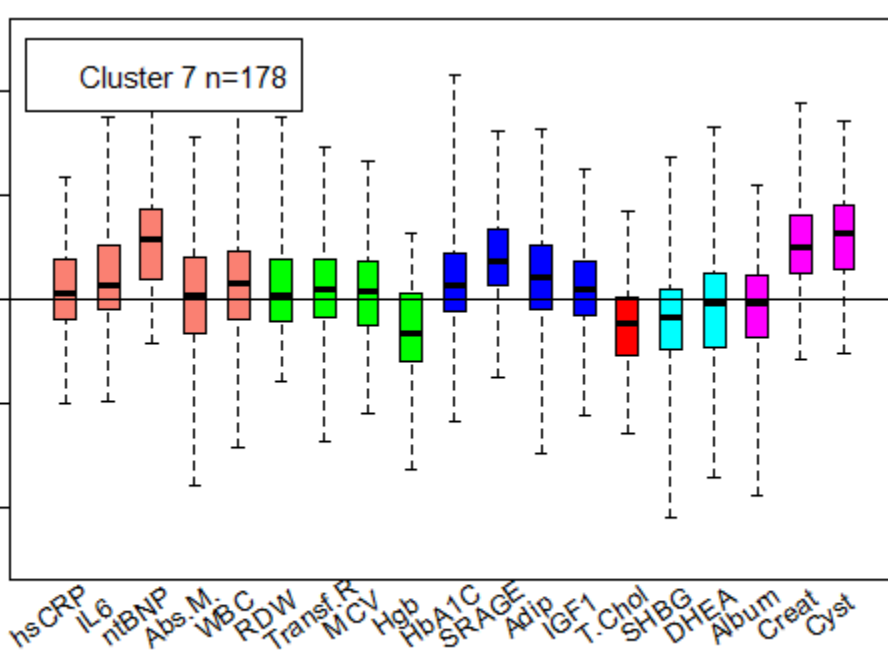
Profile 7:

Cluster:

56% born < 1935; median age 92

44% born \geq 1935; median age 62

55% females



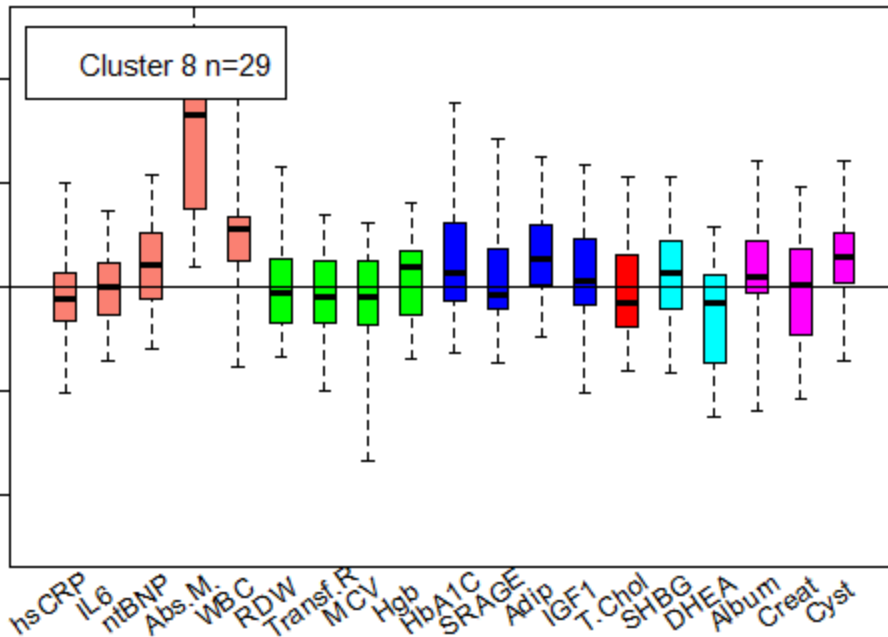
Profile 8:

Cluster:

55% born < 1935; median age 91

45% born \geq 1935; median age 58

48% females



Supplement Figure S9: Profiles 9 and 10

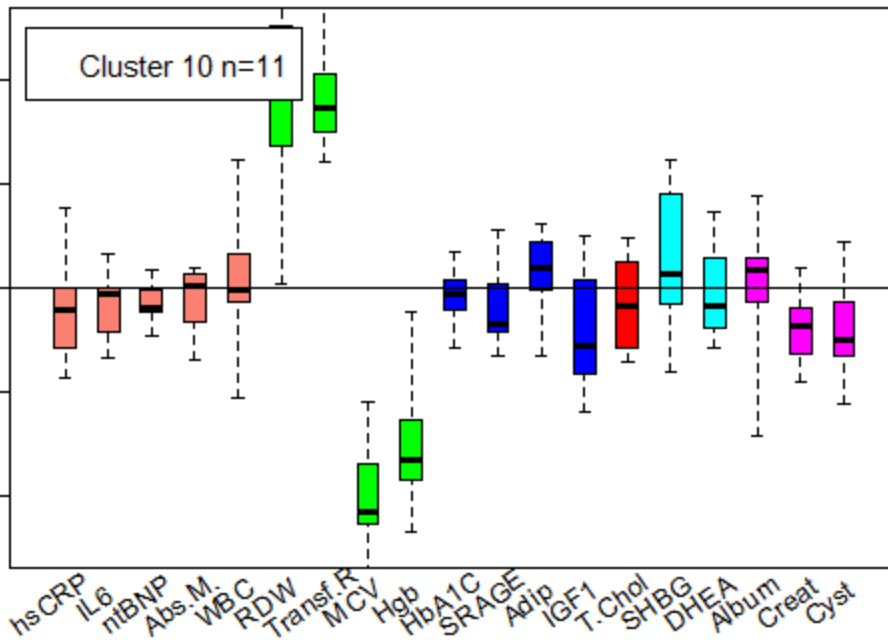
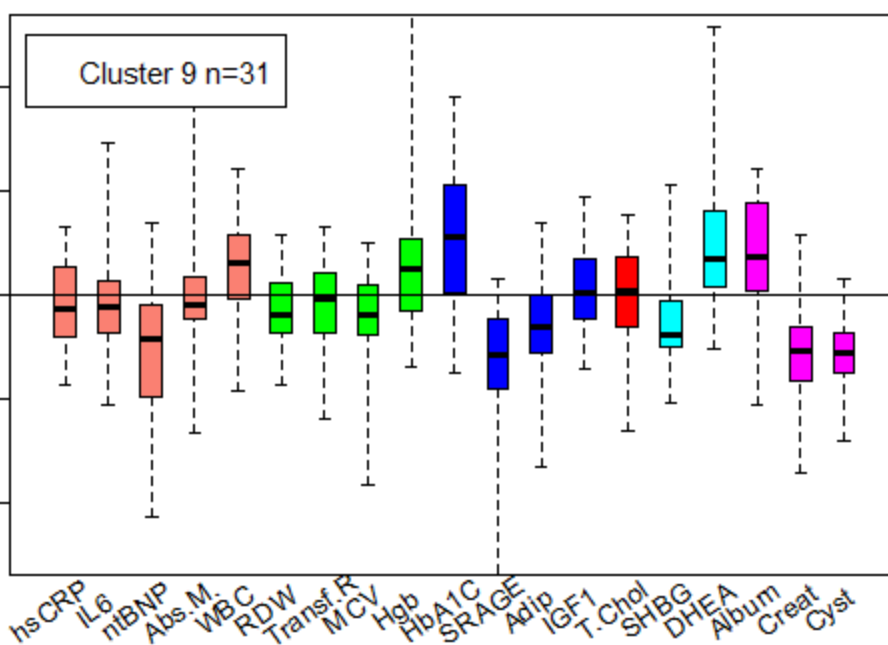
Profile 9:

Cluster:

42% born < 1935; median age 87

58% born \geq 1935; median age 64

48% females



Profile 10:

Cluster:

45% born < 1935; median age 88

55% born \geq 1935; median age 52

73% females

Supplement Figure S10: Profiles 11 and 12

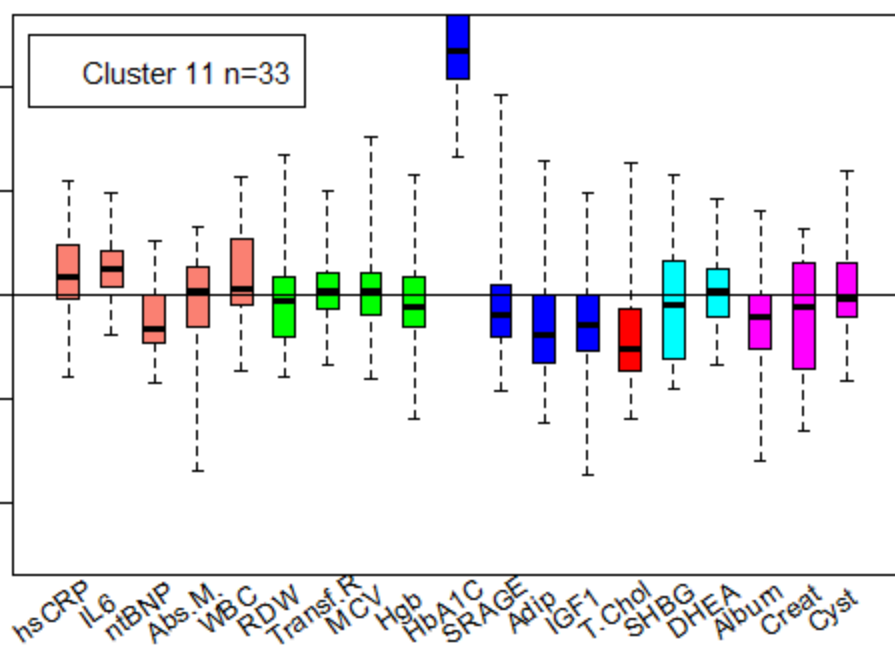
Profile 11:

Cluster:

27% born < 1935; median age 85

73% born \geq 1935; median age 59

49% females



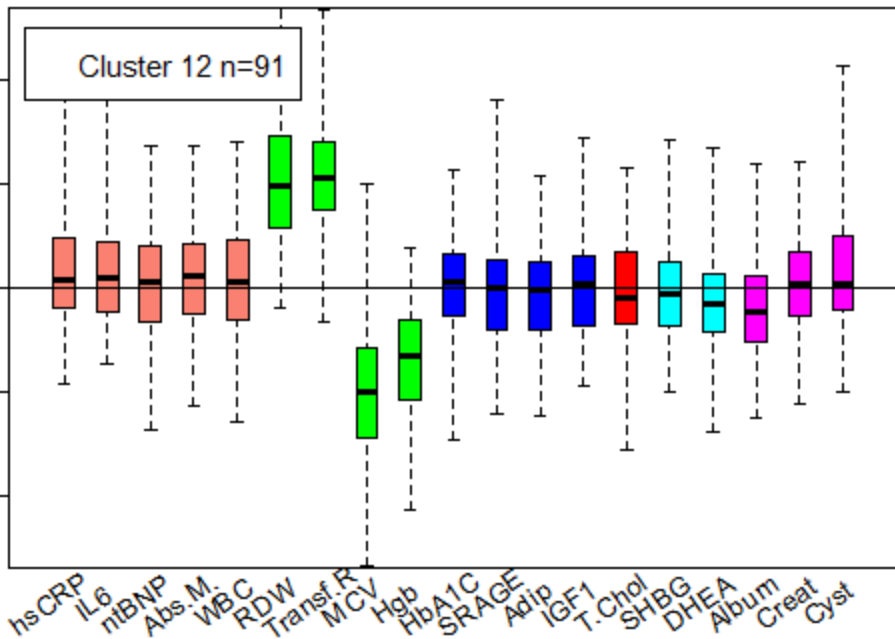
Profile 12:

Cluster:

42% born < 1935; median age 92

58% born \geq 1935; median age 59

54% females



Supplement Figure S11: Profiles 13 and 14

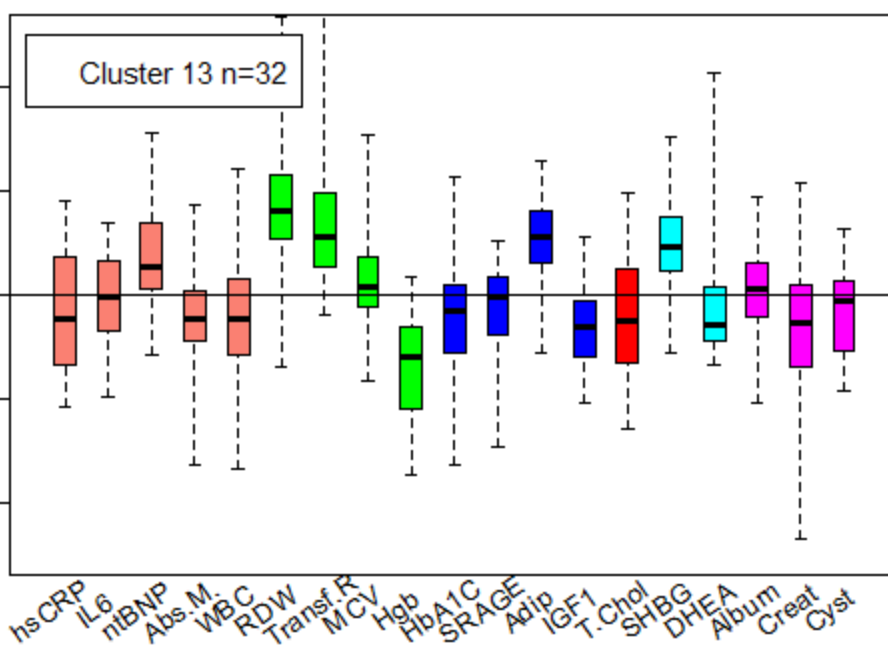
Profile 13:

Cluster:

66% born < 1935; median age 89

34% born \geq 1935; median age 57

55% females



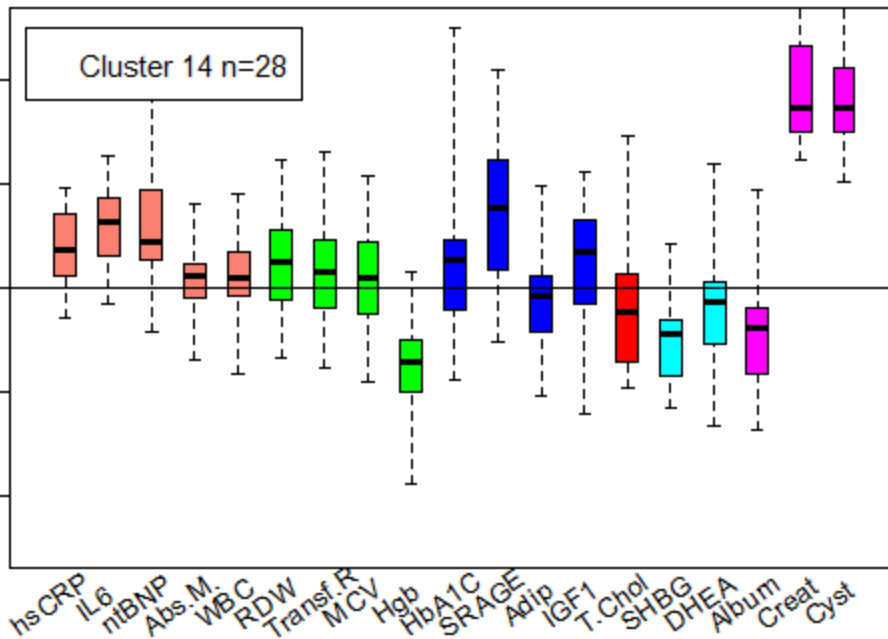
Profile 14:

Cluster:

57% born < 1935; median age 91

43% born \geq 1935; median age 57

61% females



Supplement Figure S12: Profiles 15 and 16

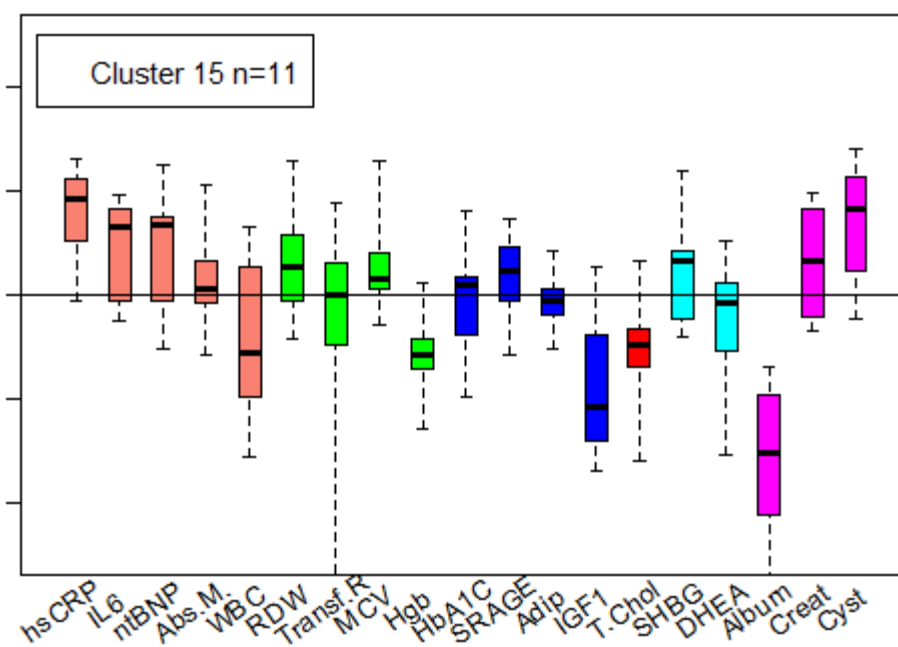
Profile 15:

Cluster:

55% born < 1935; median age 89

45% born \geq 1935; median age 63

73% females



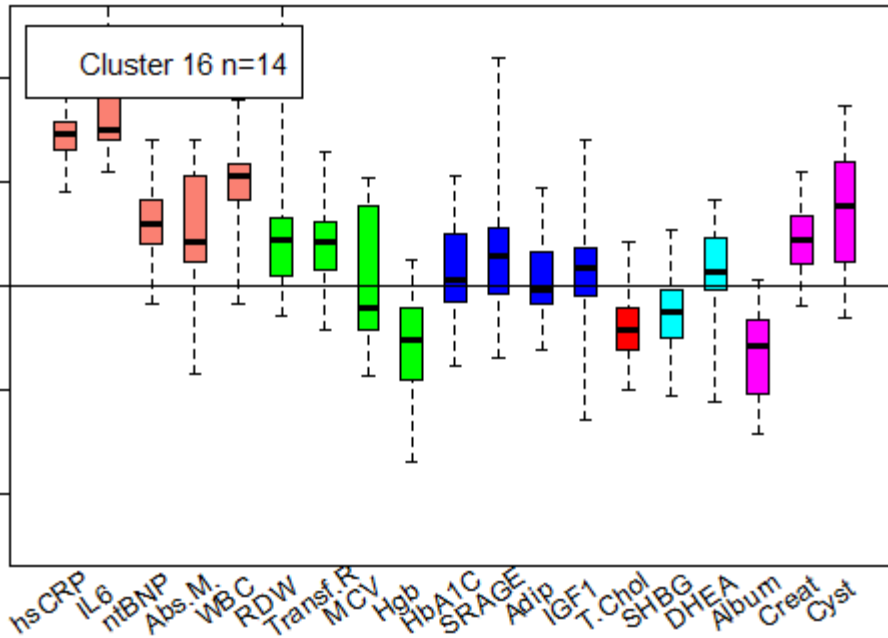
Profile 16:

Cluster:

64% born < 1935; median age 92

36% born \geq 1935; median age 63

29% females



Supplement Figure S13: Profiles 17 and 18

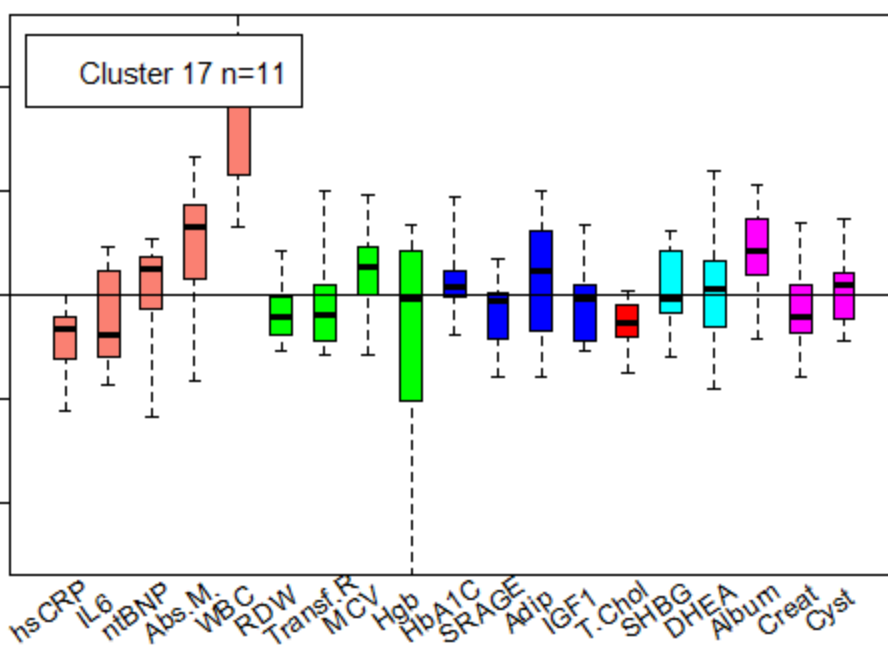
Profile 17:

Cluster:

55% born < 1935; median age 90

45% born \geq 1935; median age 61

46% females



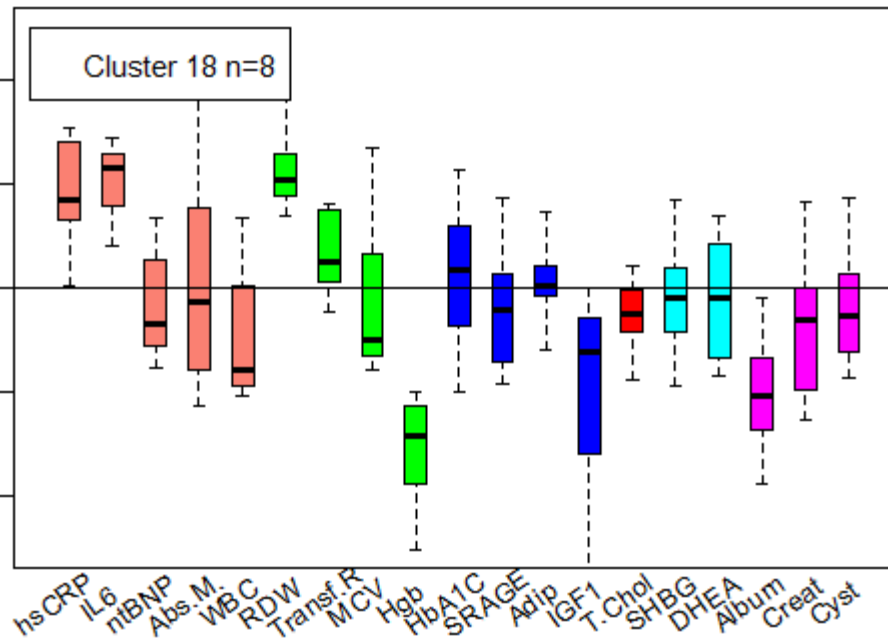
Profile 18:

Cluster:

50% born < 1935; median age 98

40% born \geq 1935; median age 55

75% females



Supplement Figure S14: Profiles 19 and 20

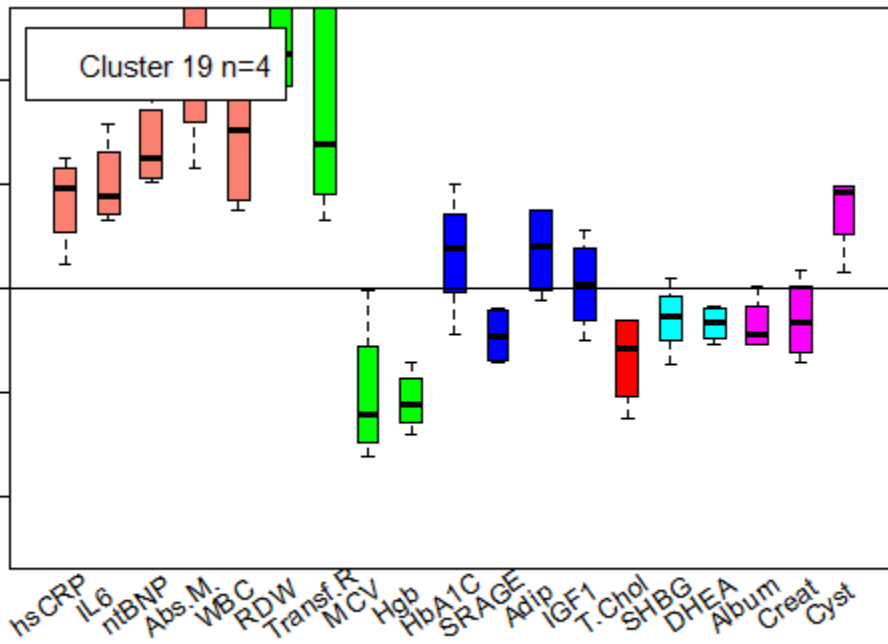
Profile 19:

Cluster:

50% born < 1935; median age 79

60% born \geq 1935; median age 59

25% females



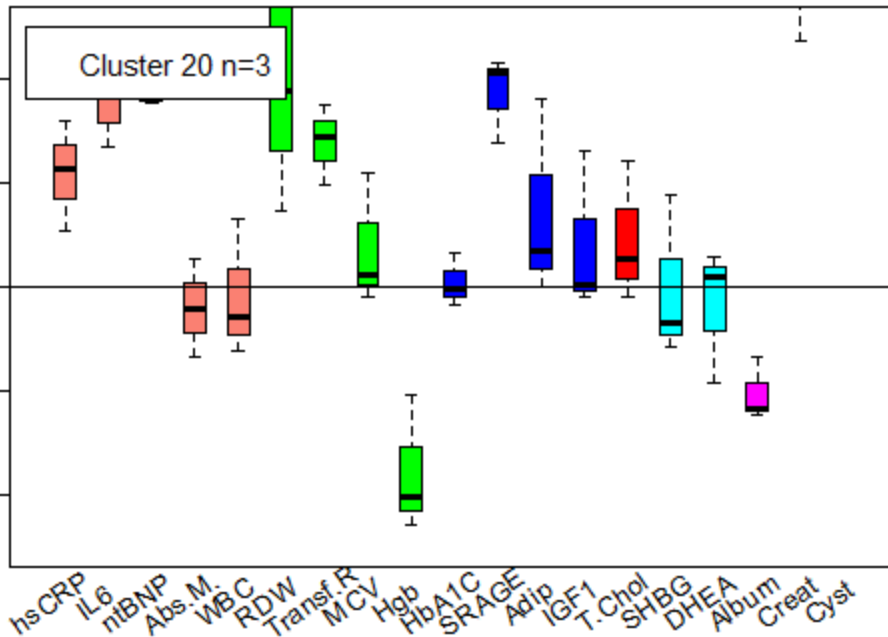
Profile 20:

Cluster:

0% born < 1935

100% born \geq 1935; median age 57

0% females



Supplement Figure S15: Profiles 21 and 22

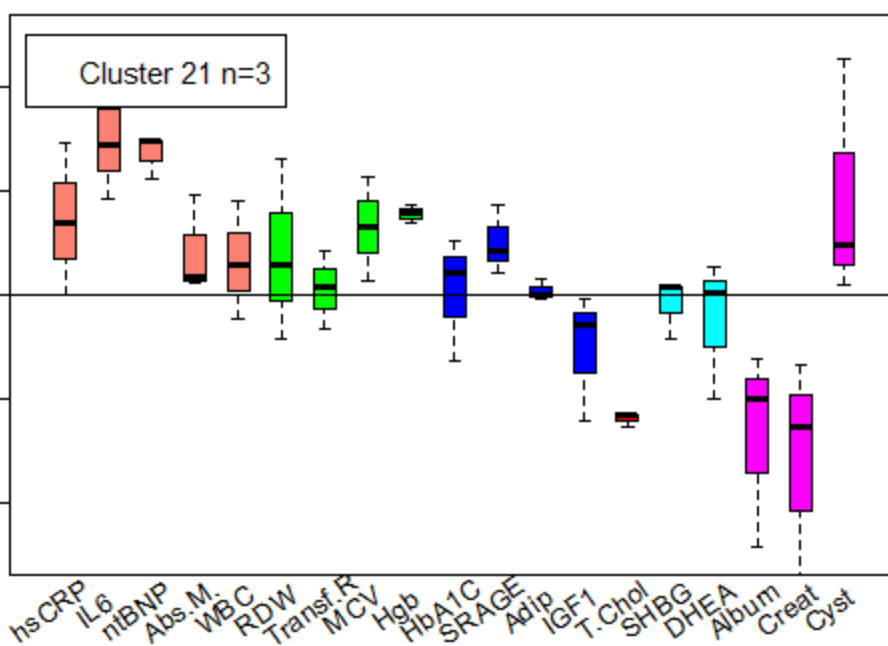
Profile 21:

Cluster:

0% born < 1935;

100% born \geq 1935; median age 62

67% females



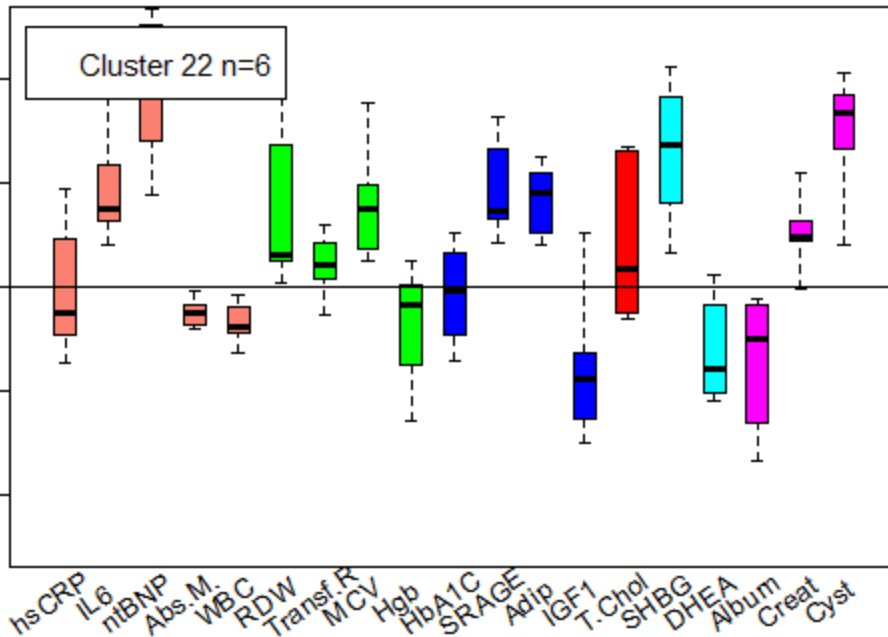
Profile 22:

Cluster:

0% born < 1935;

10% born \geq 1935; median age 62

50% females

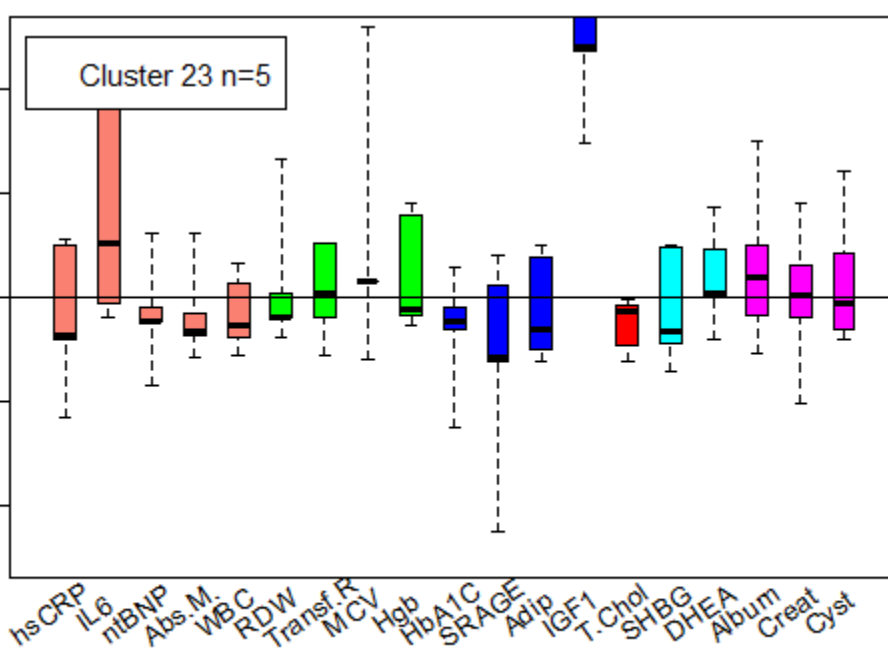


Supplement Figure S16: Profiles 23 and 24

Profile 23:

Cluster:

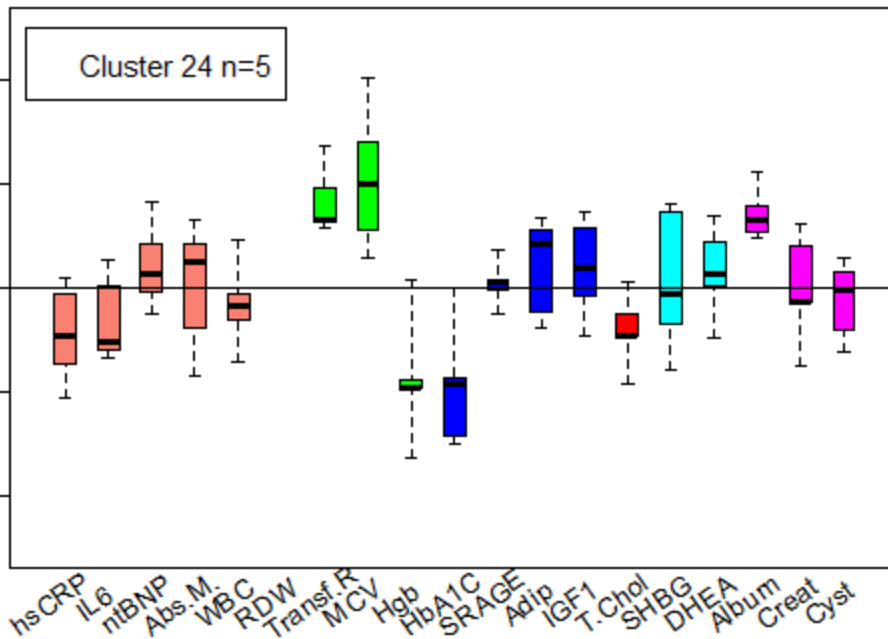
60% born < 1935; median age 90
40% born \geq 1935; median age 62
80% females



Profile 24:

Cluster:

80% born < 1935; median age 92
20% born \geq 1935; median age 60
80% females

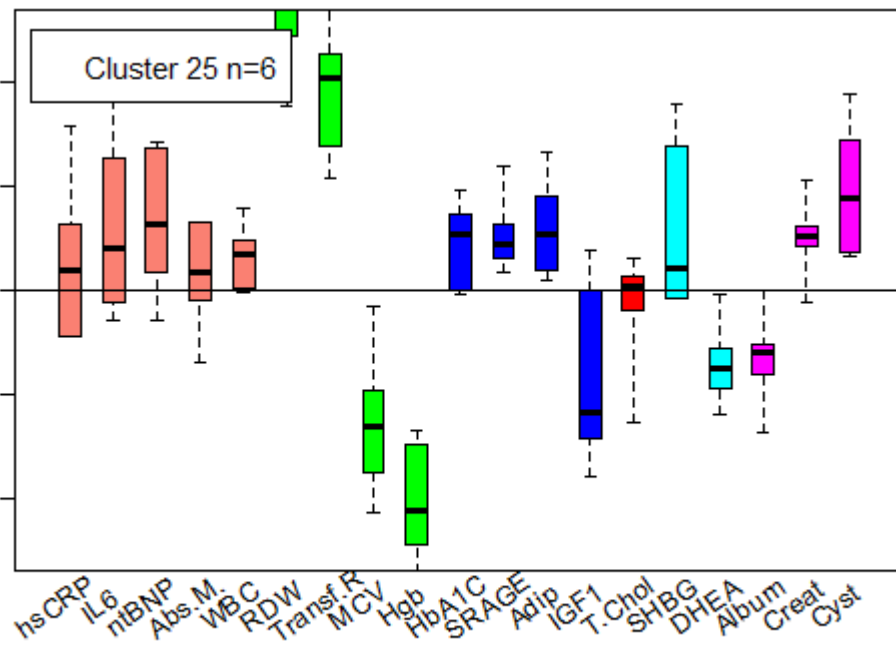


Supplement Figure S17: Profiles 25 and 26

Profile 25:

Cluster:

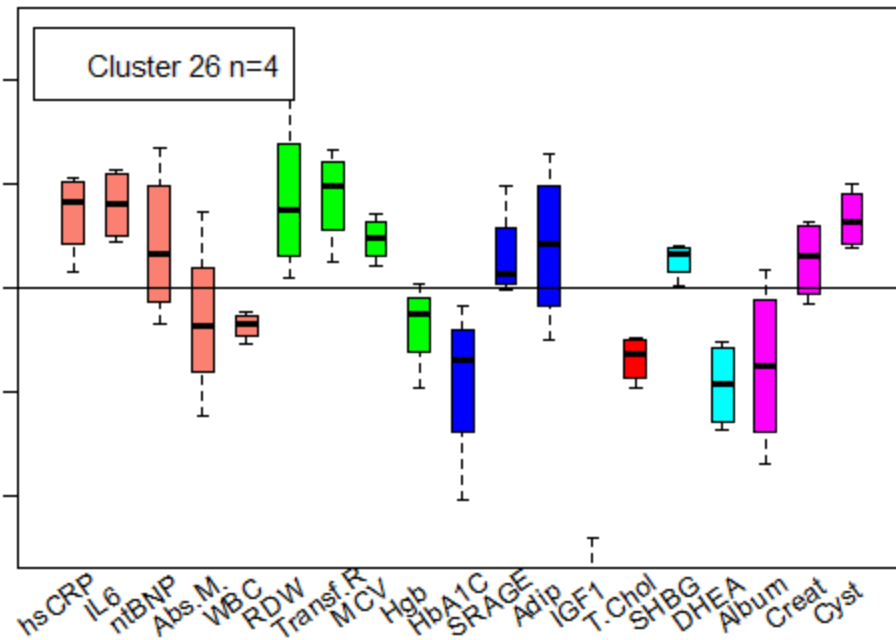
16% born < 1935; median age 84
84% born \geq 1935; median age 64
50% females



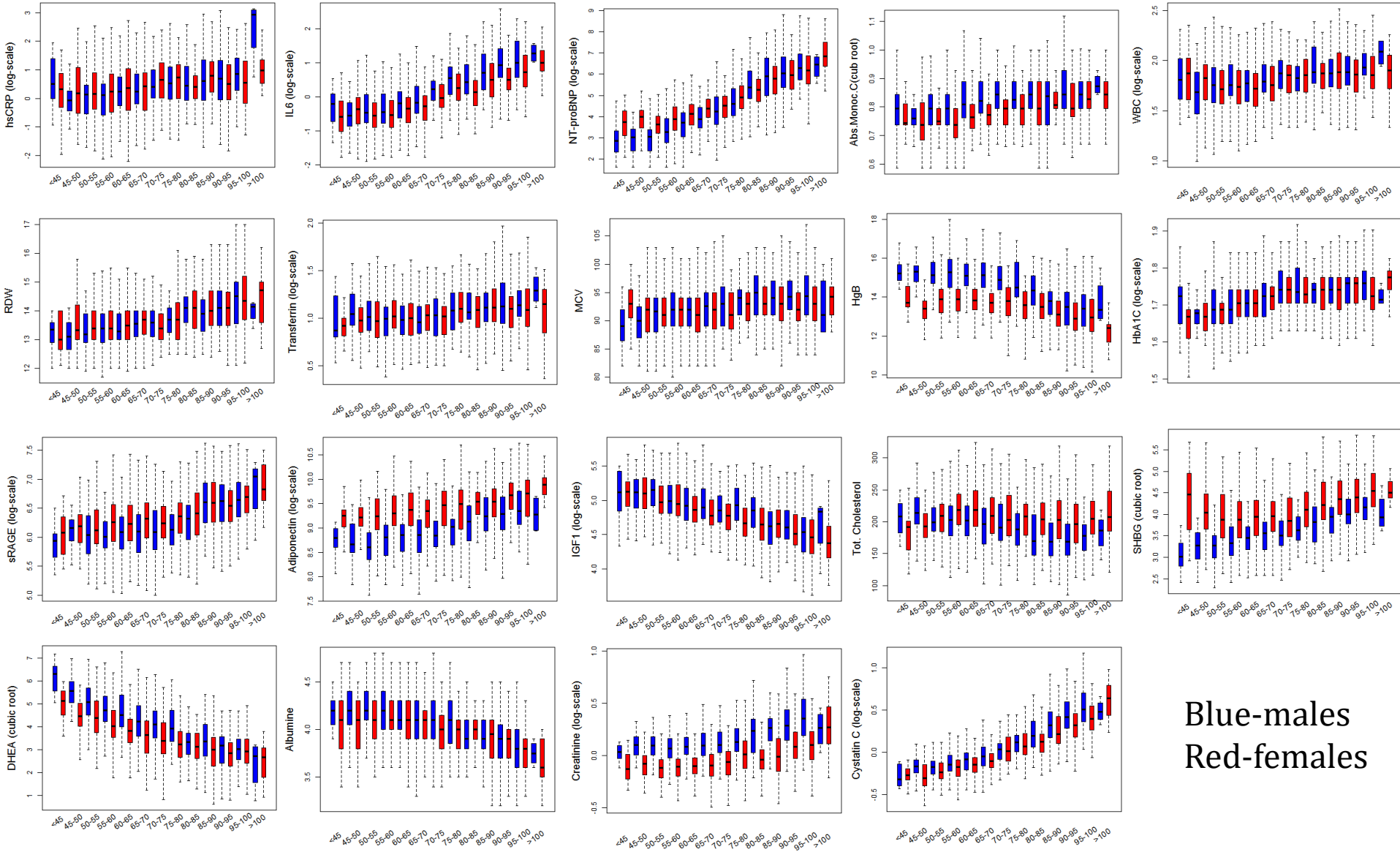
Profile 26:

Cluster:

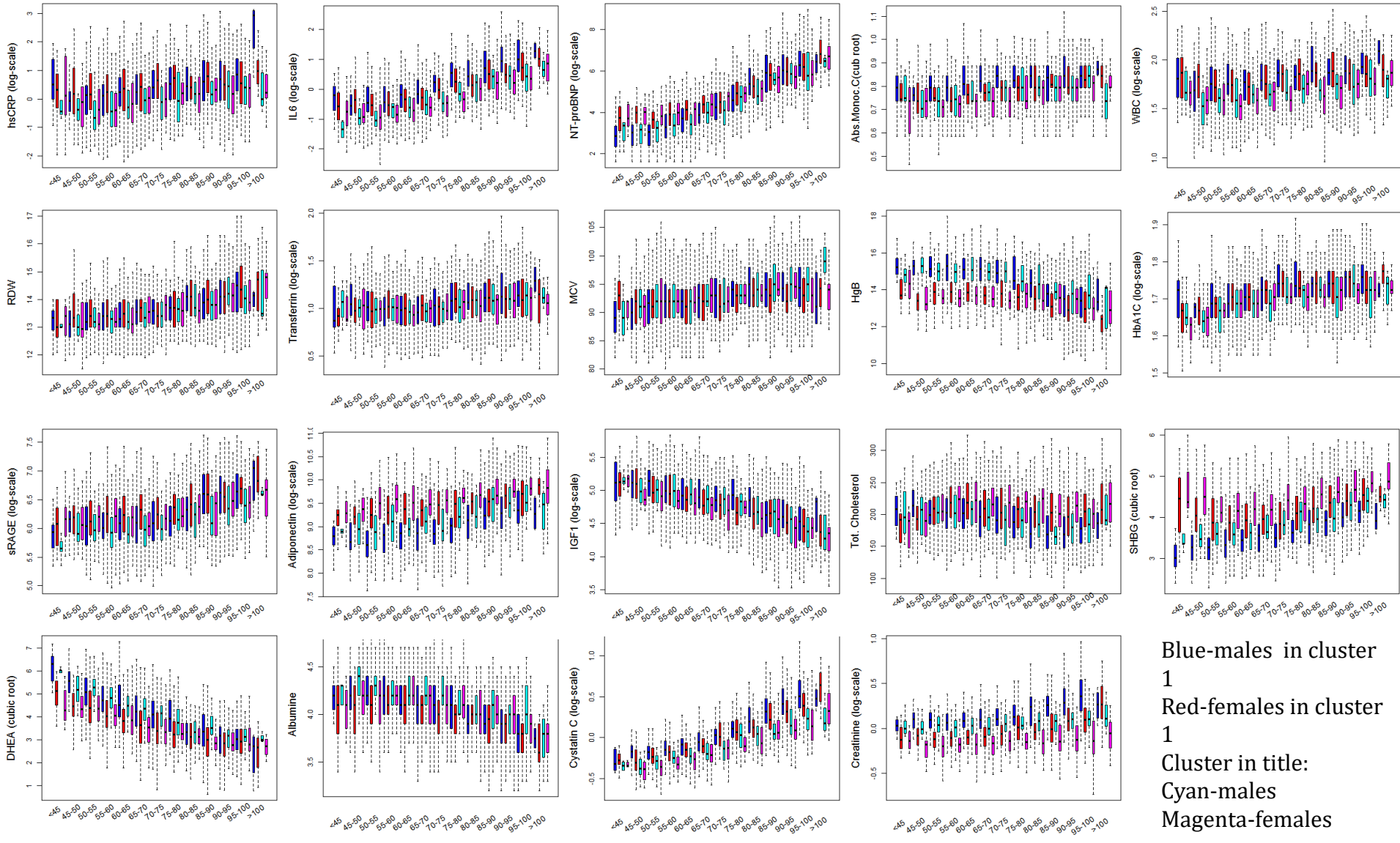
50% born < 1935; median age 97
50% born \geq 1935; median age 57
50% females



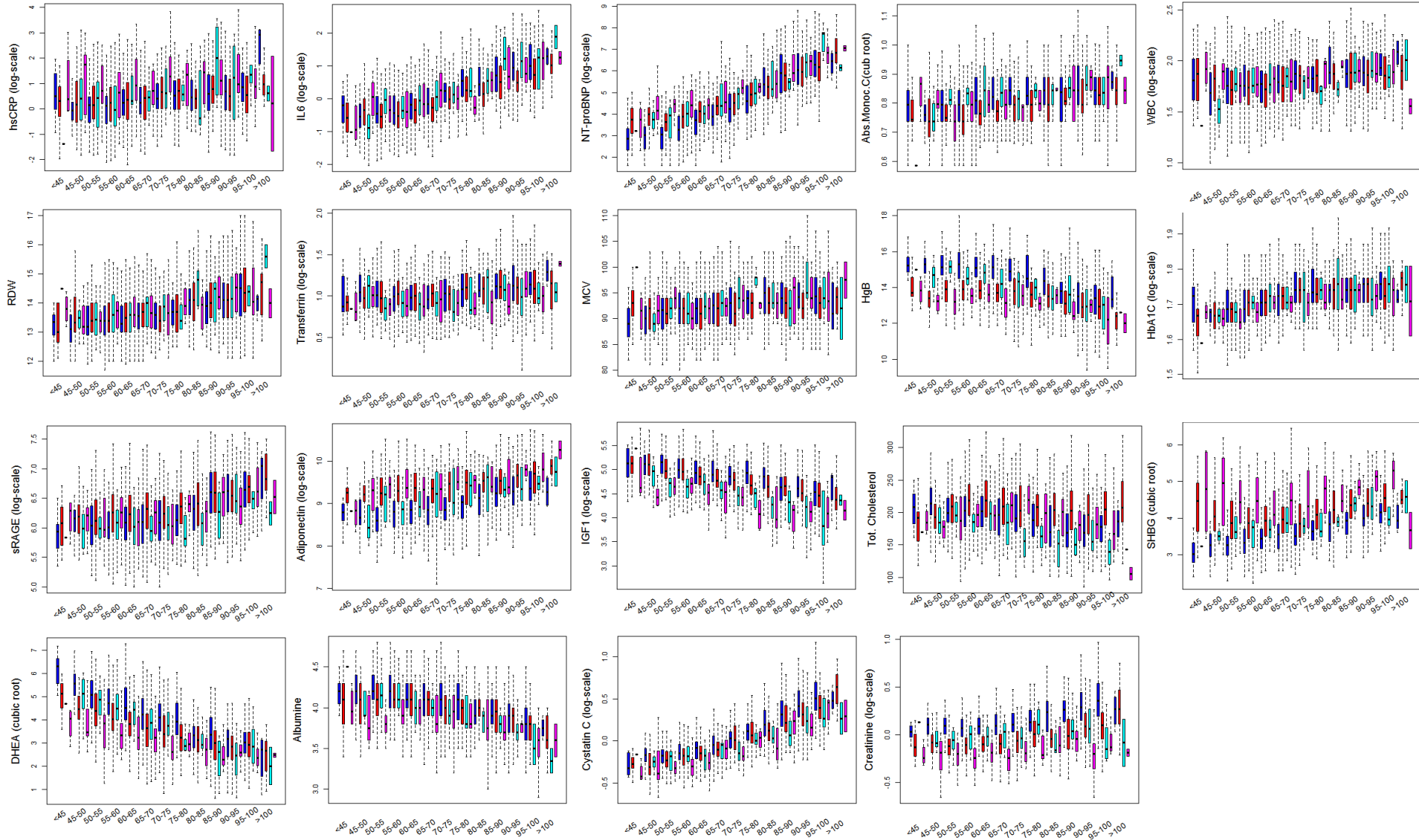
Supplement Figure S18_a: Referent Cluster



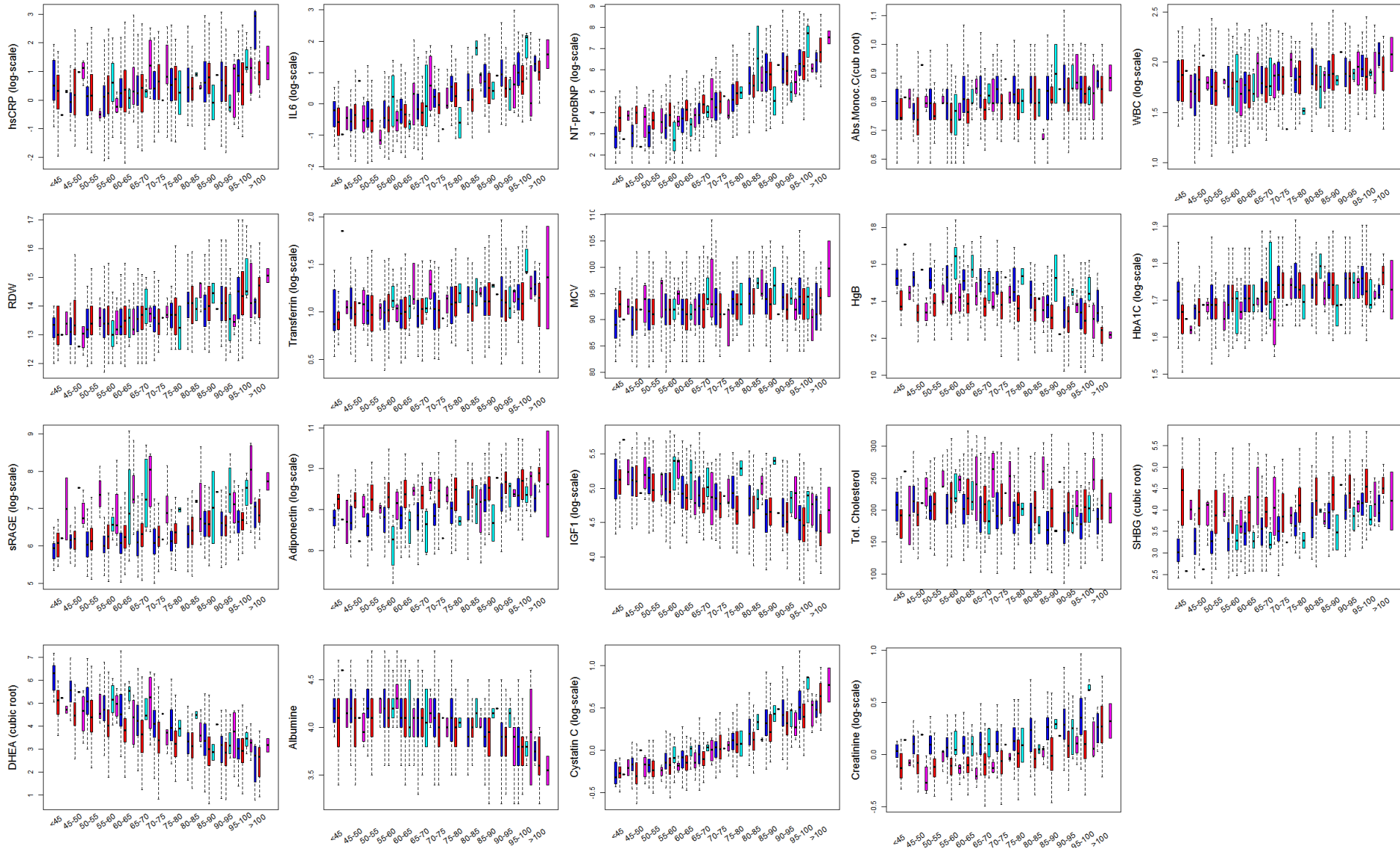
Supplement Figure S18_b: Cluster 2



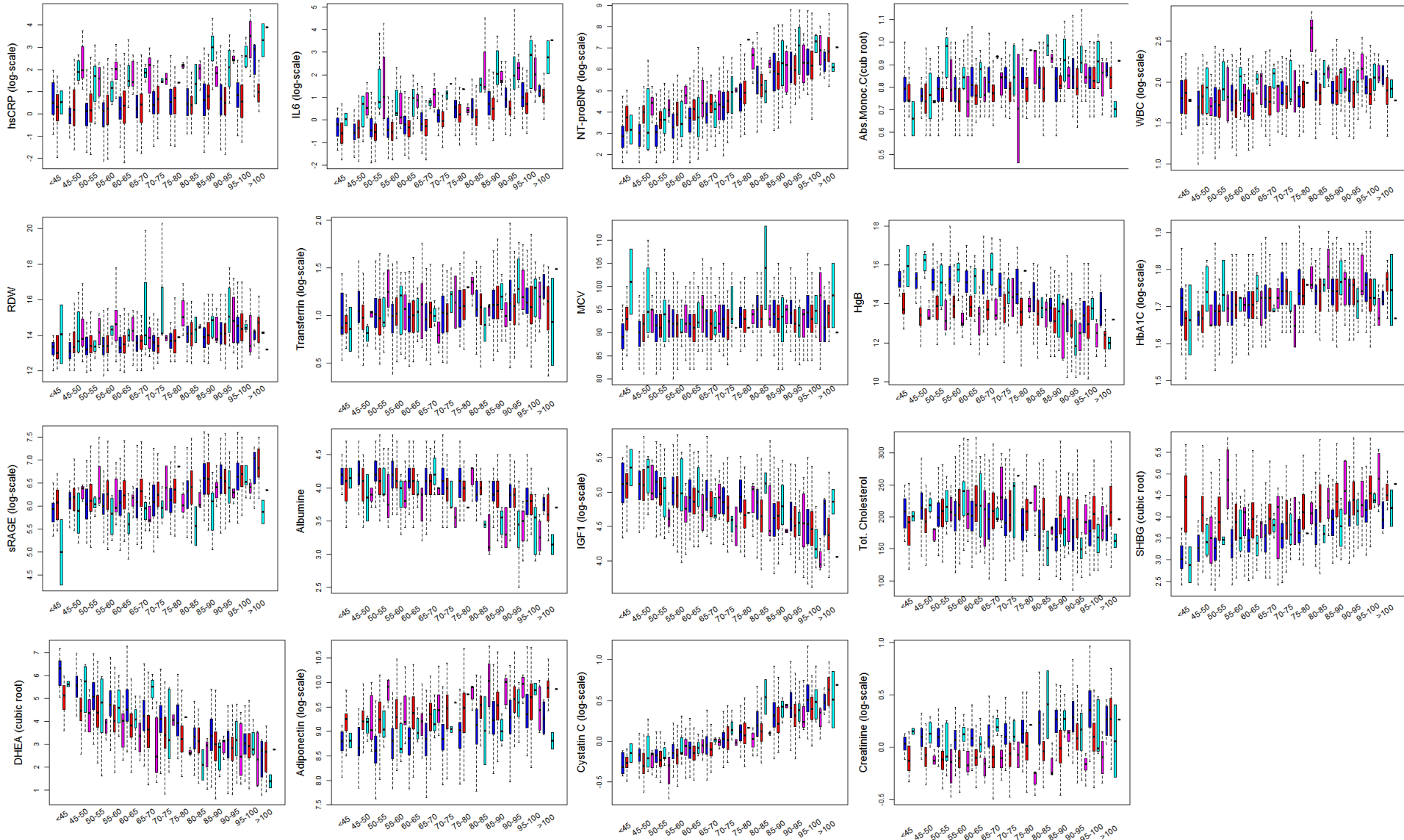
Supplement Figure S18_c: Cluster 3



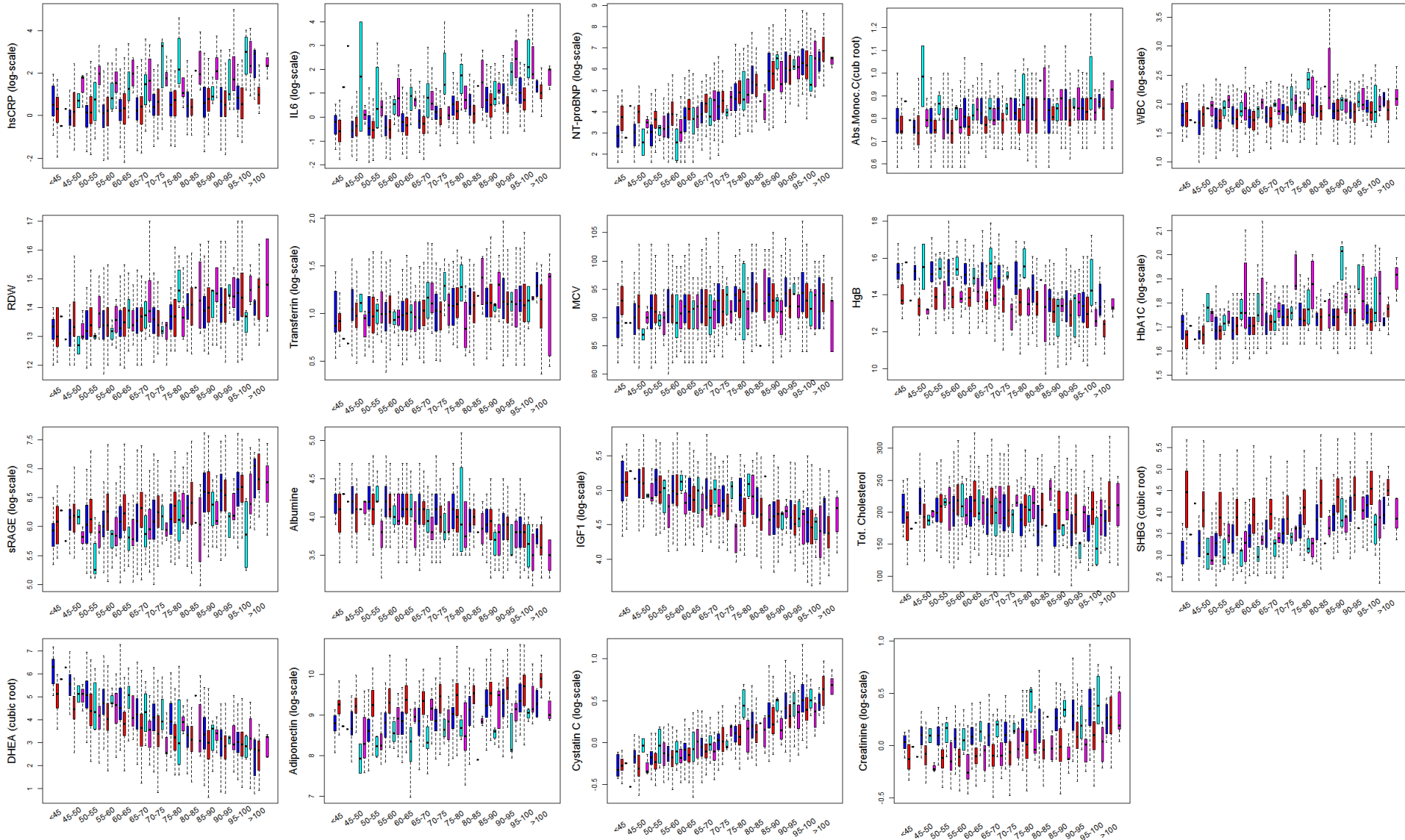
Supplement Figure S18_d: Cluster 4



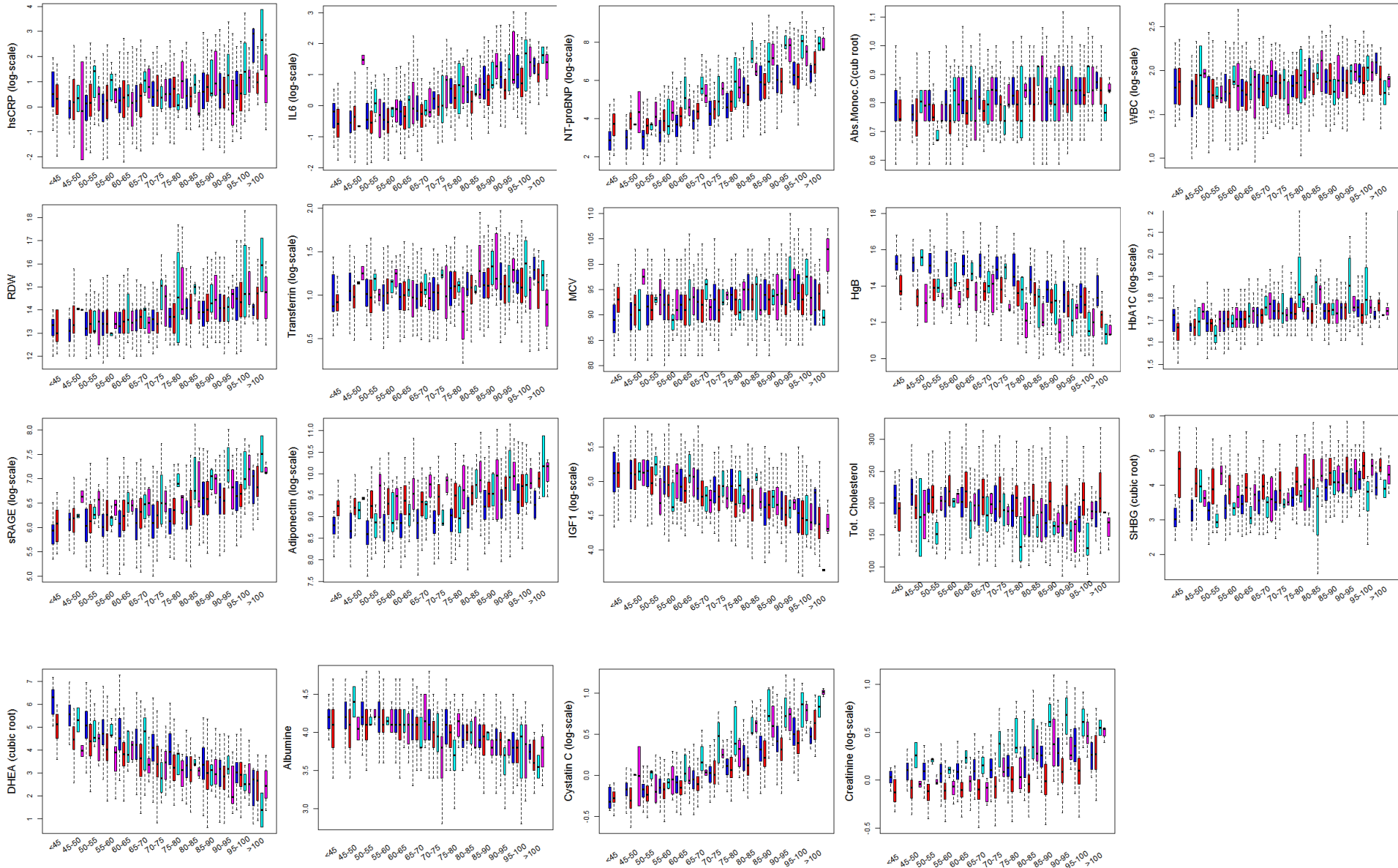
Supplement Figure S18_e: Cluster 5



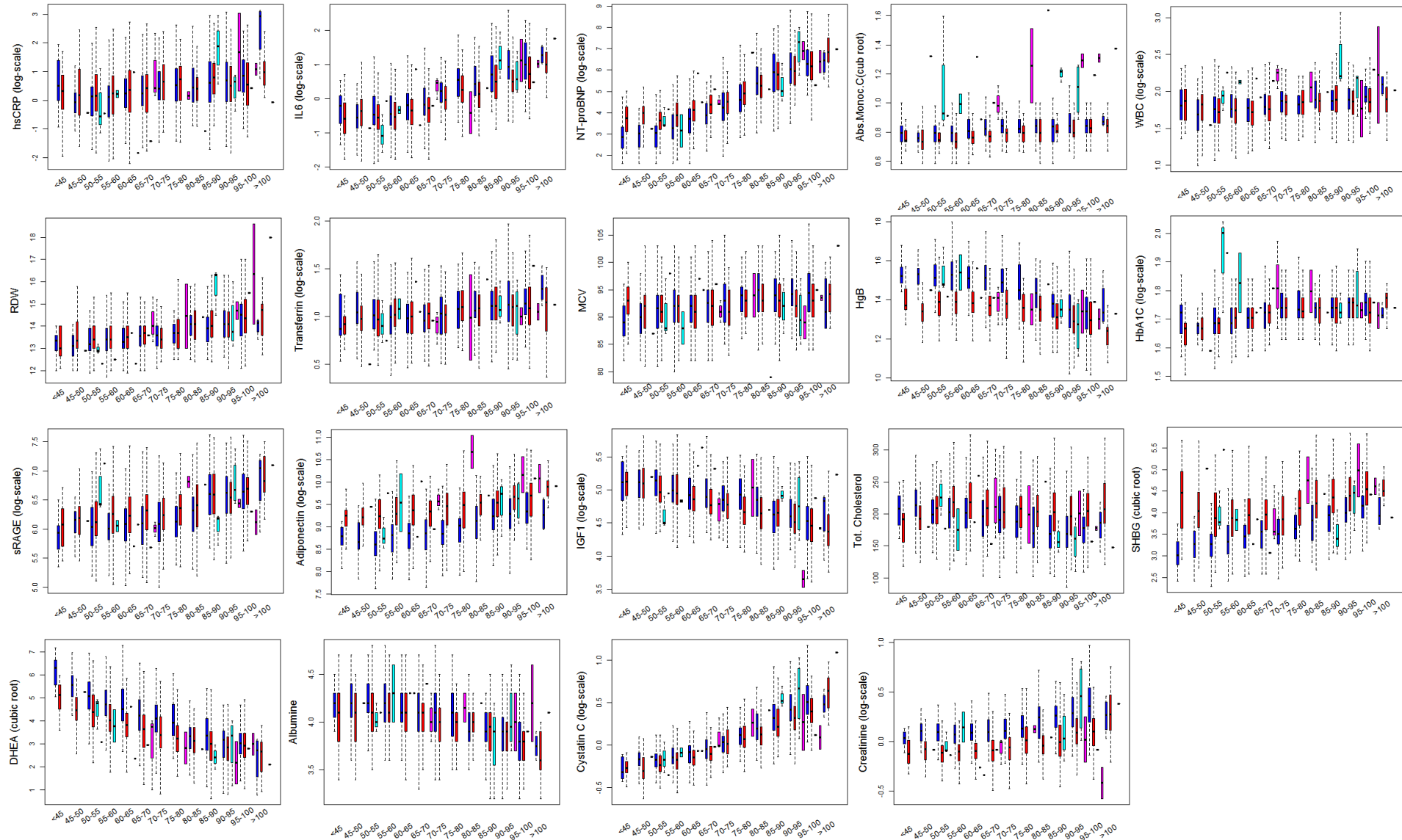
Supplement Figure S18_f: Cluster 6



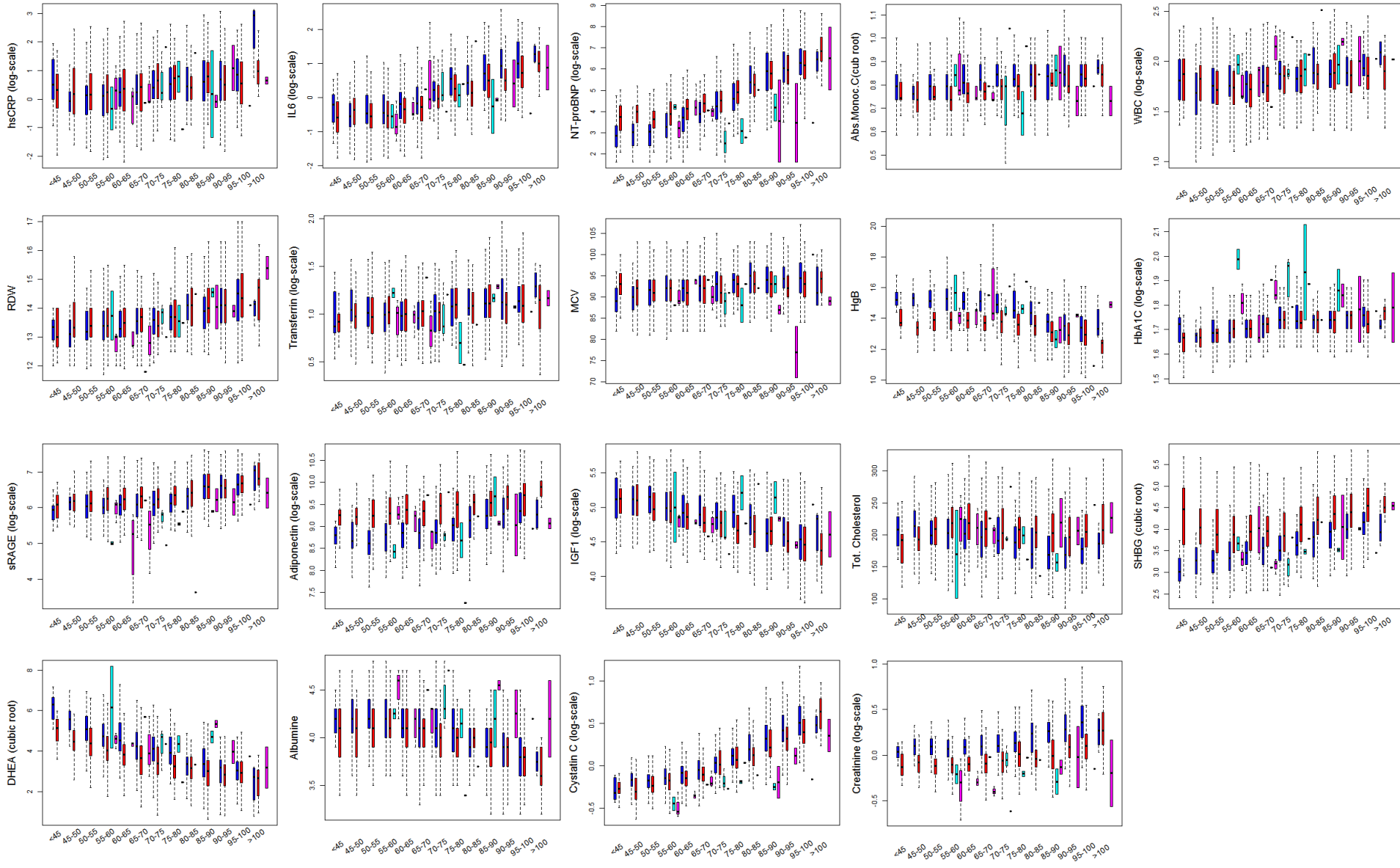
Supplement Figure S18_g: Cluster 7



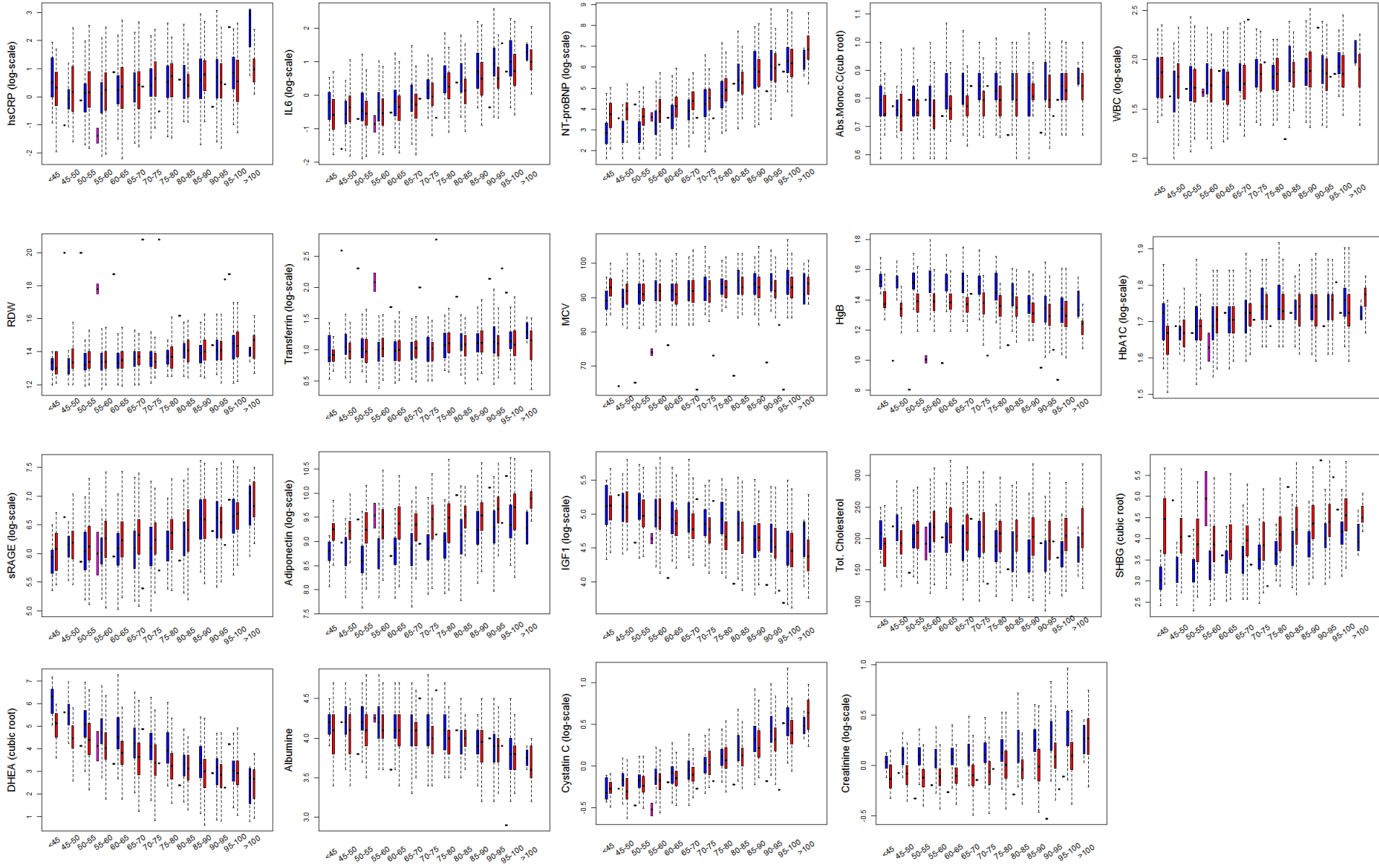
Supplement Figure S18_h: Cluster 8



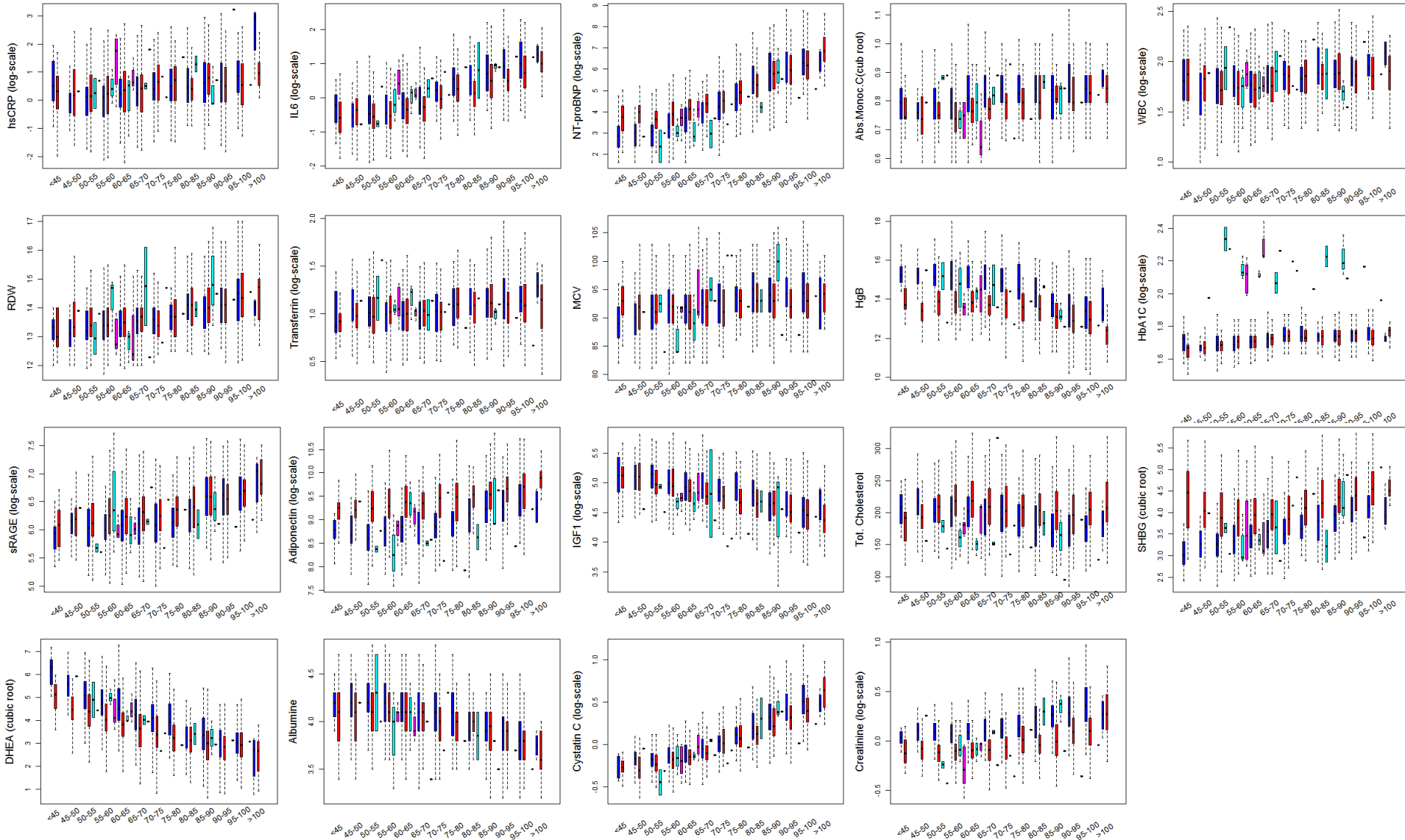
Supplement Figure S18_k: Cluster 9



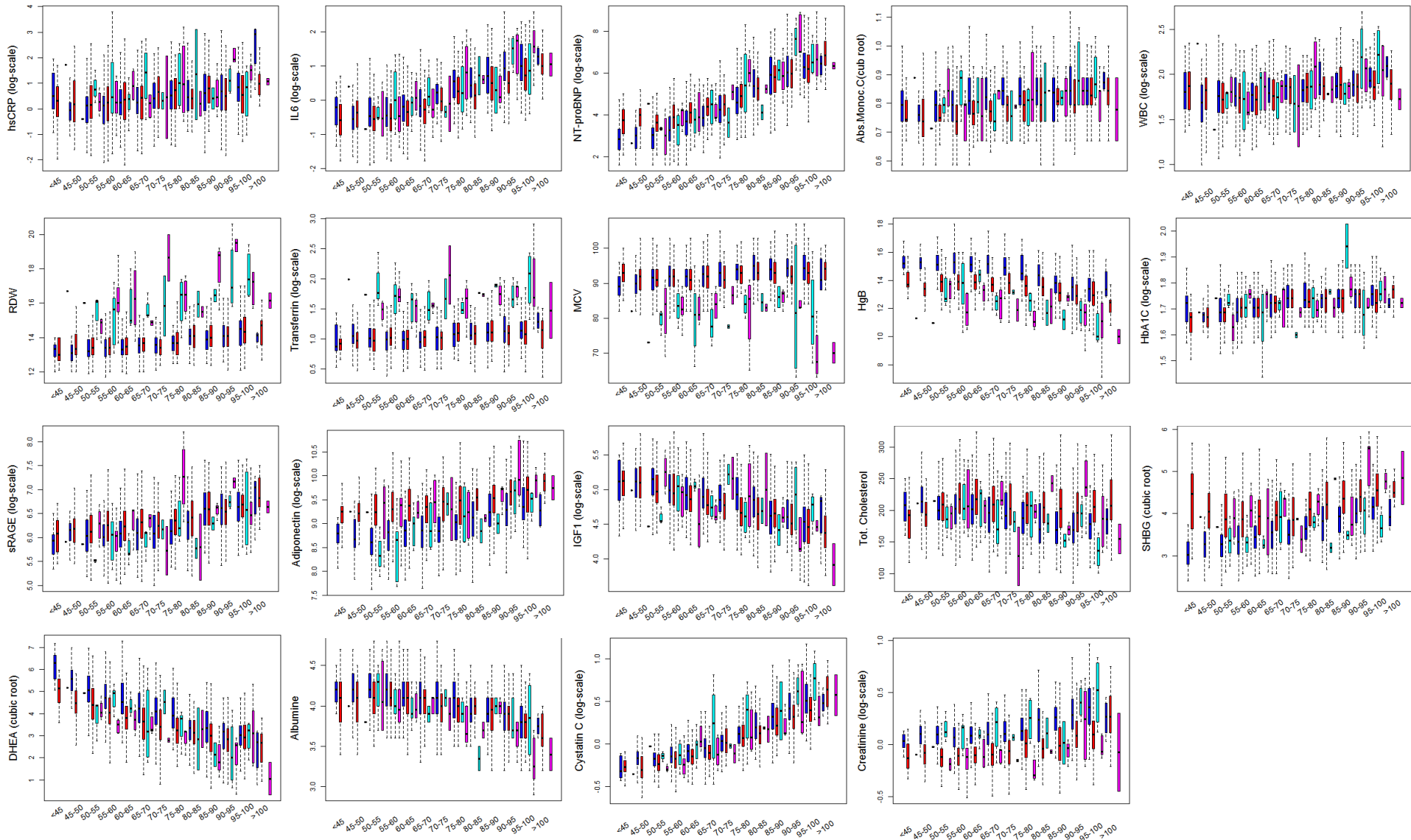
Supplement Figure S18_j: Cluster 10



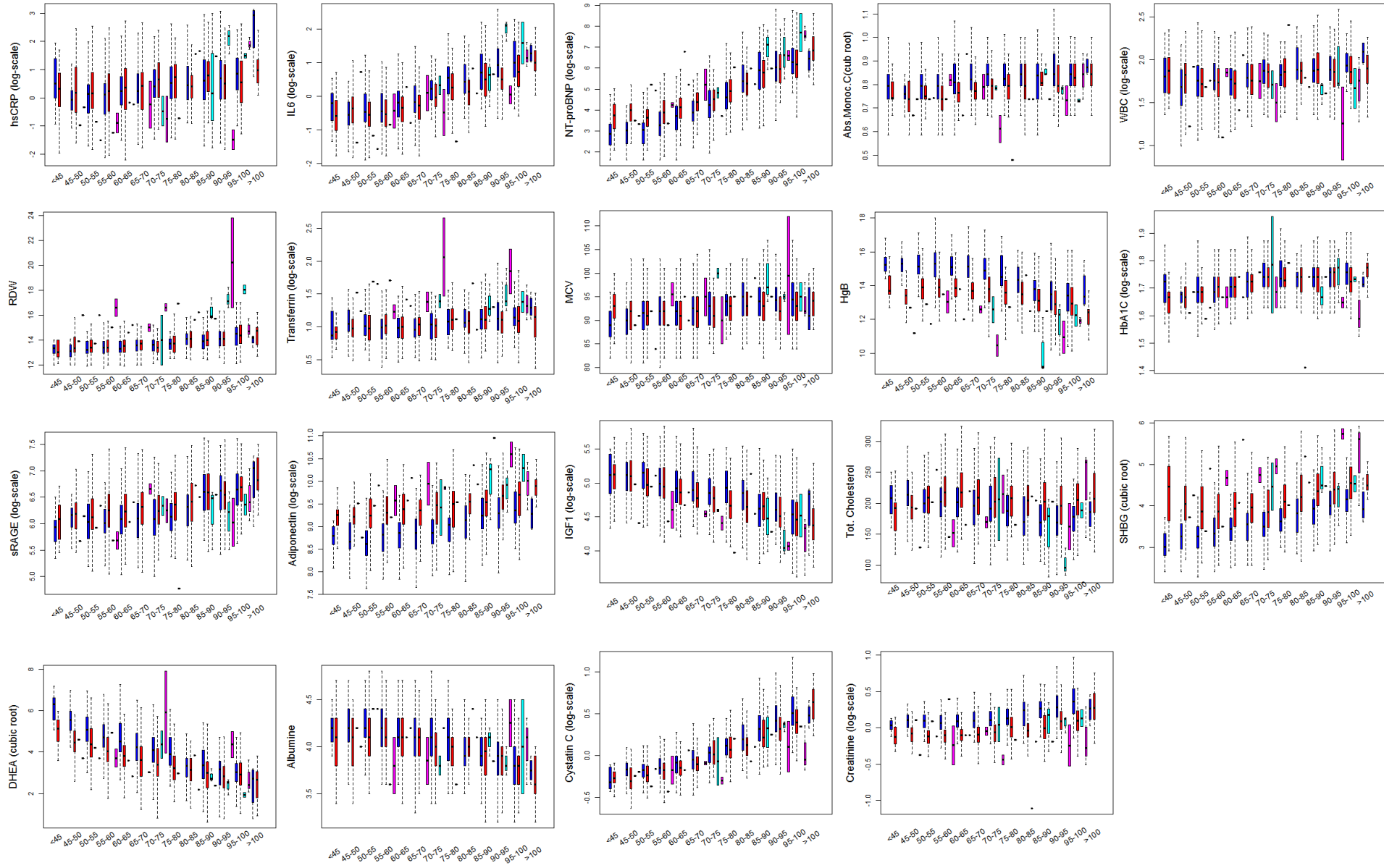
Supplement Figure S18_k: Cluster 11



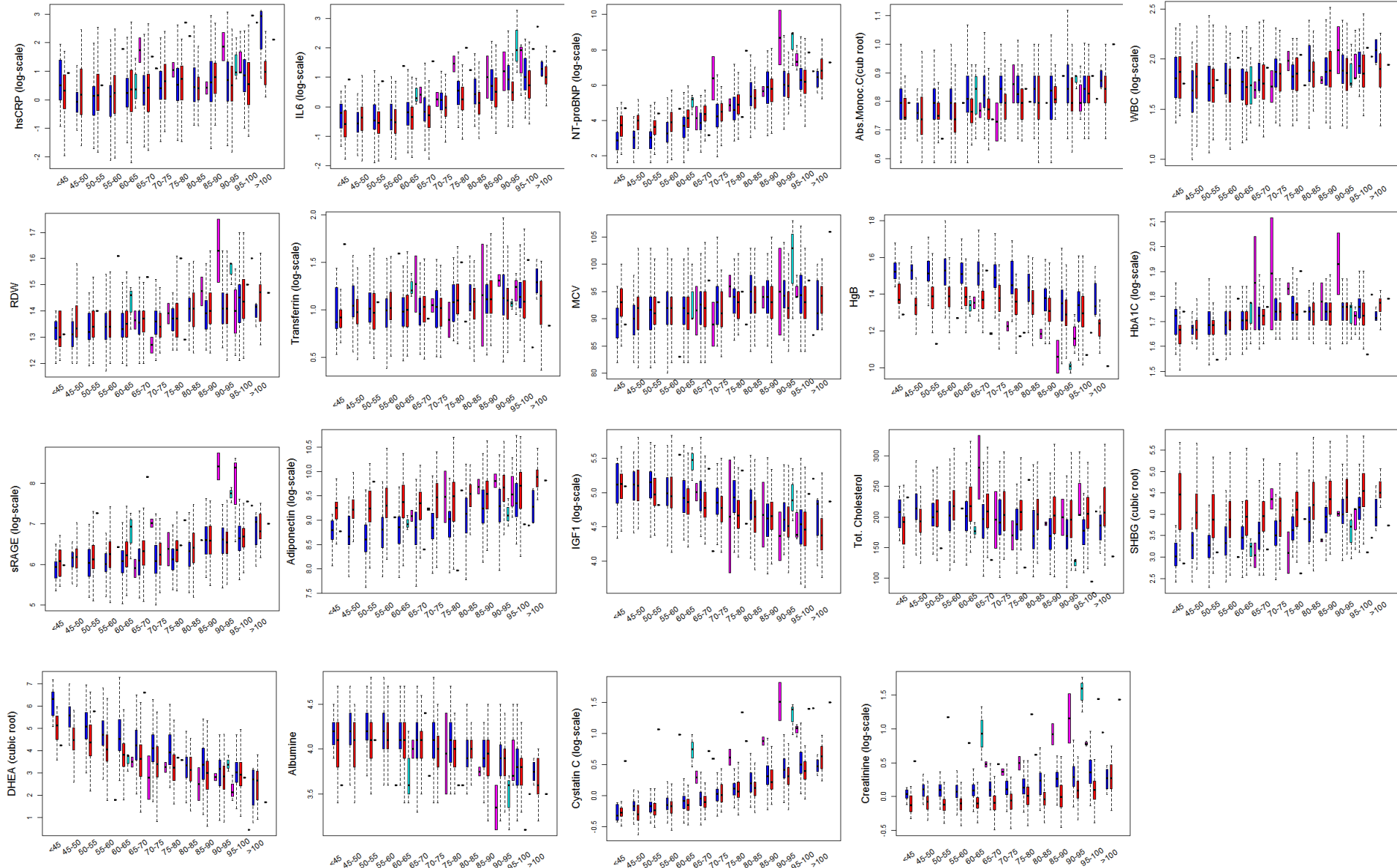
Supplement Figure S18_m: Cluster 12



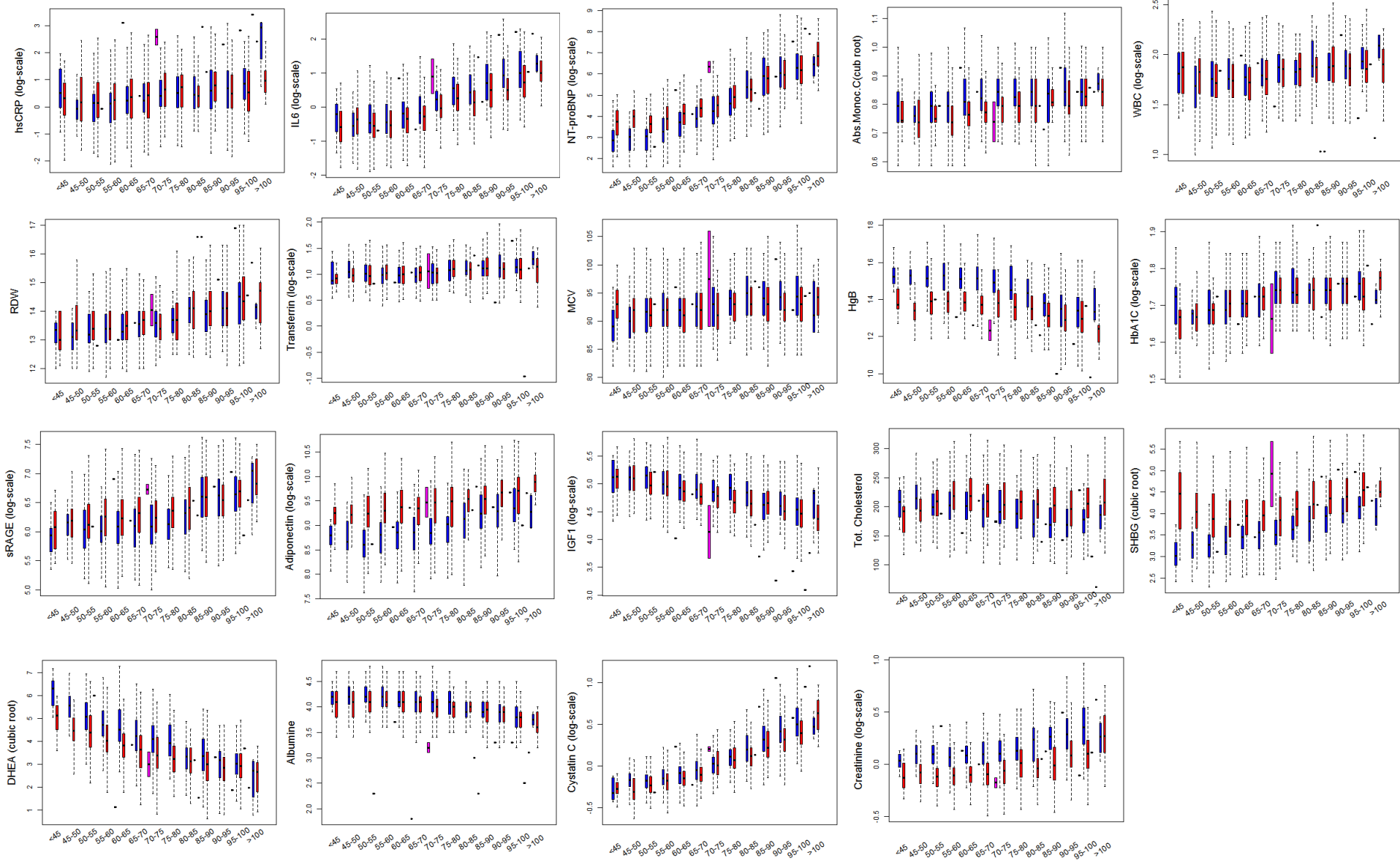
Supplement Figure S18_n: Cluster 13



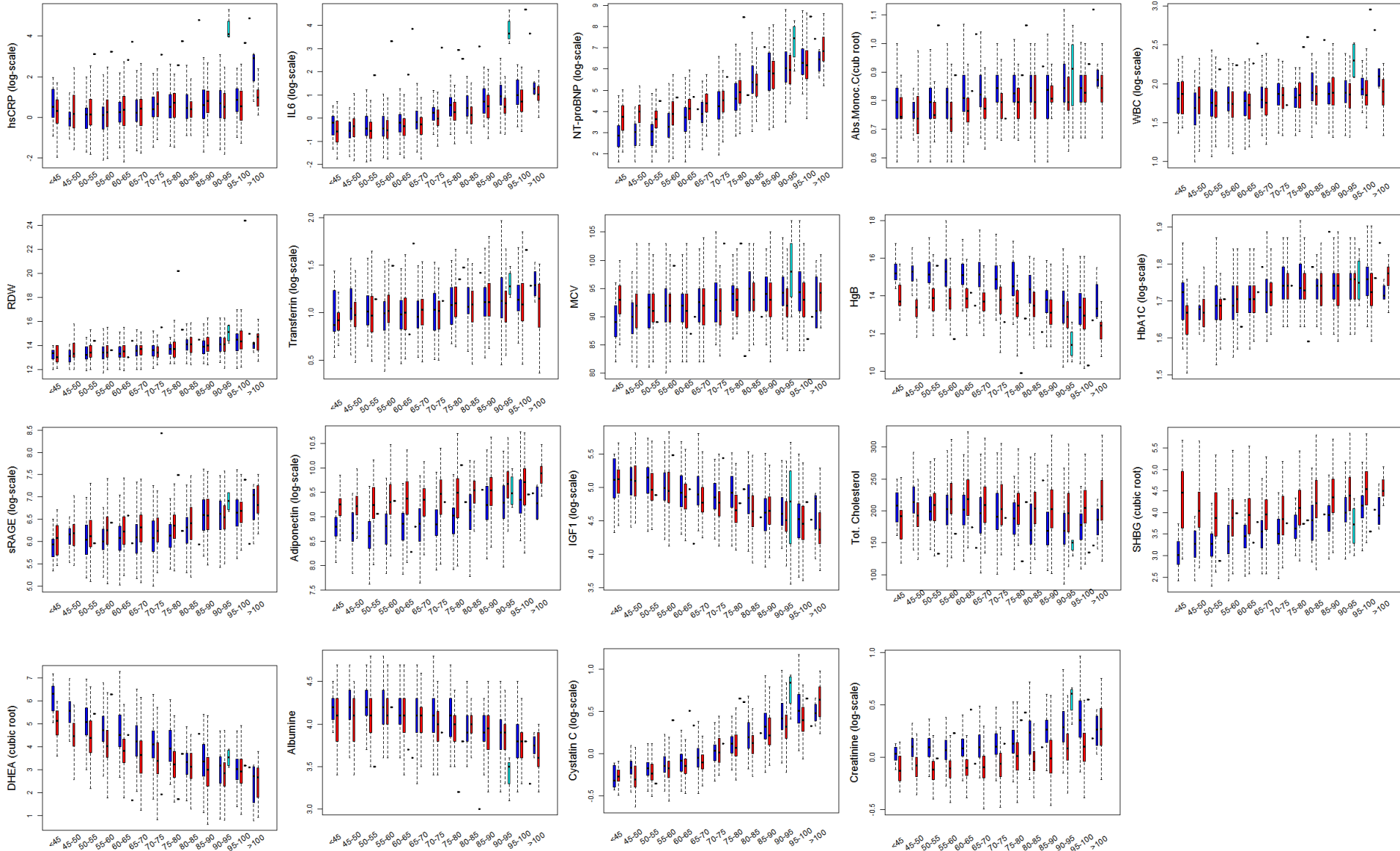
Supplement Figure S18_0: Cluster 14



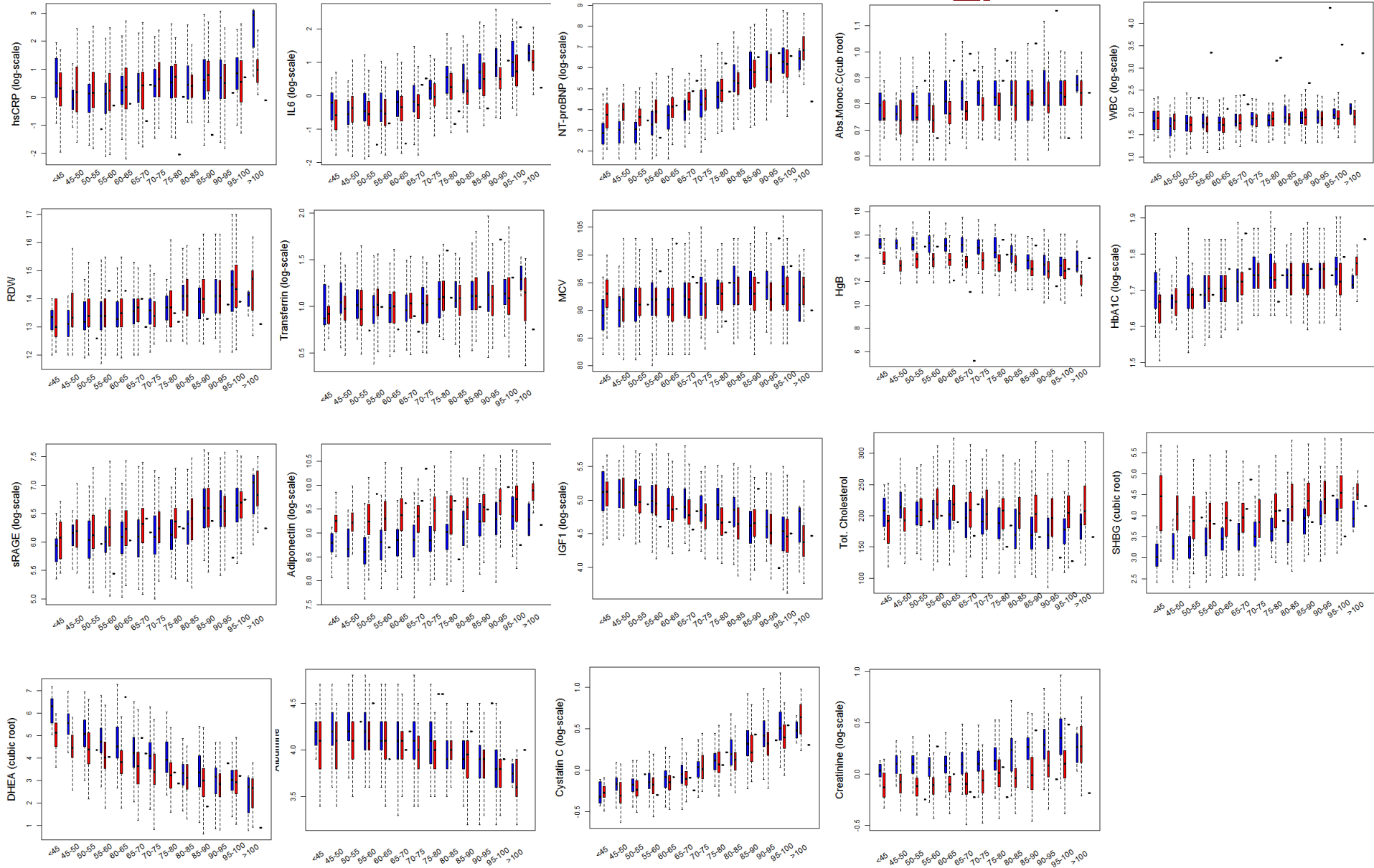
Supplement Figure S18_p: Cluster 15



Supplement Figure S18_p: Cluster 16

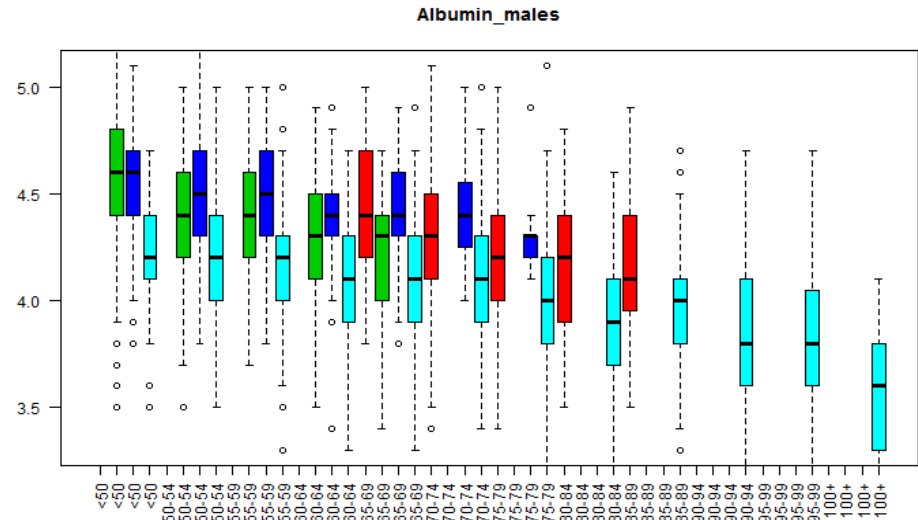
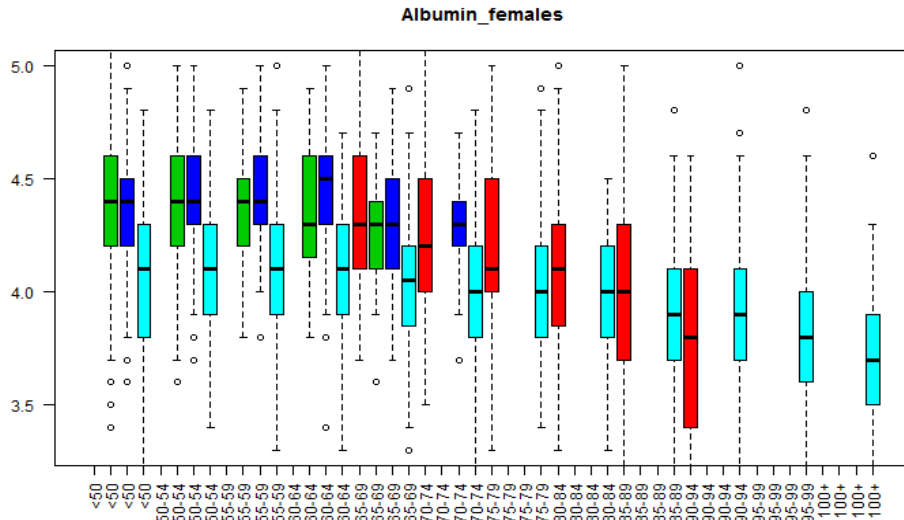


Supplement Figure S18_p: Cluster 17

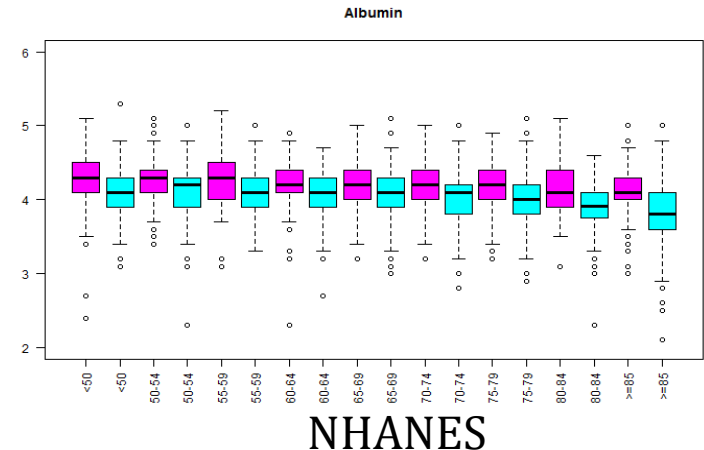


Supplement Figure S19: Example of Lab-to-Lab Bias in Albumin Measurements

Red=FHS-gen-1 ex20;
Green = FHS-gen 2 exam 7;
Blue= FHS-gen3 - ex1;
Pale blue = LLFS;
Normal range 3.5; 5.4



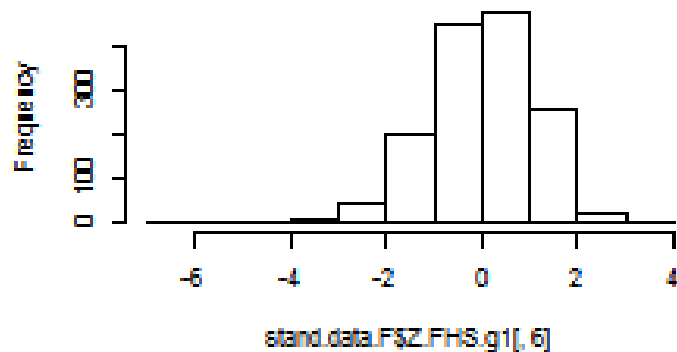
Measurements in FHS tend to be systematically higher at younger ages (batch effect), and converge at older ages. So in older LLFS participants, we see higher than expected albumin. The batch effect is consistent with what we see in NHANES



Supplement Figure S20-a: Externally Standardized Adiponectin

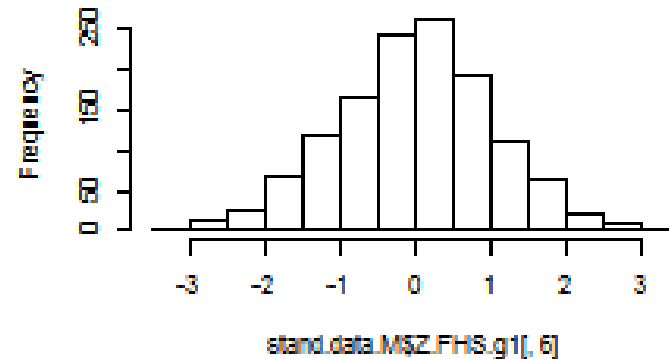
Females

Histogram of stand.data.F\$Z.FHS.g1[, 6]

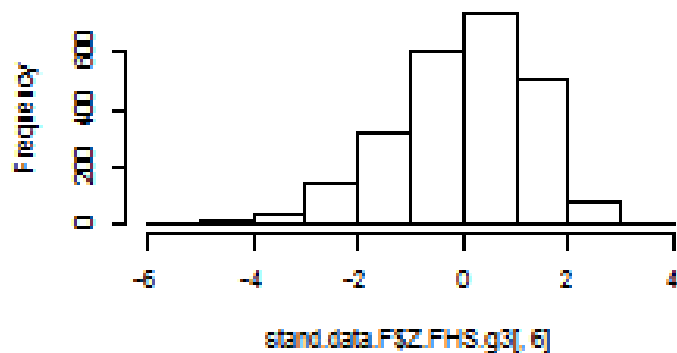


Males

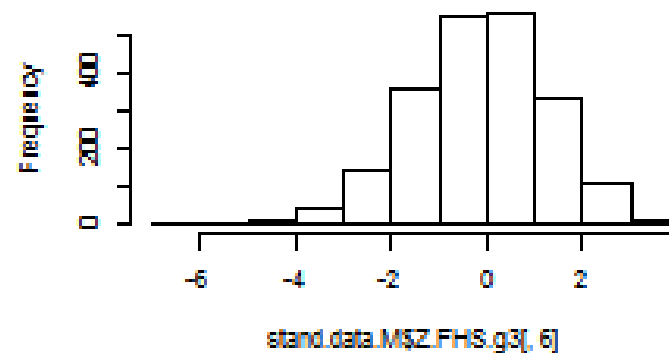
Histogram of stand.data.M\$Z.FHS.g1[, 6]



Histogram of stand.data.F\$Z.FHS.g3[, 6]

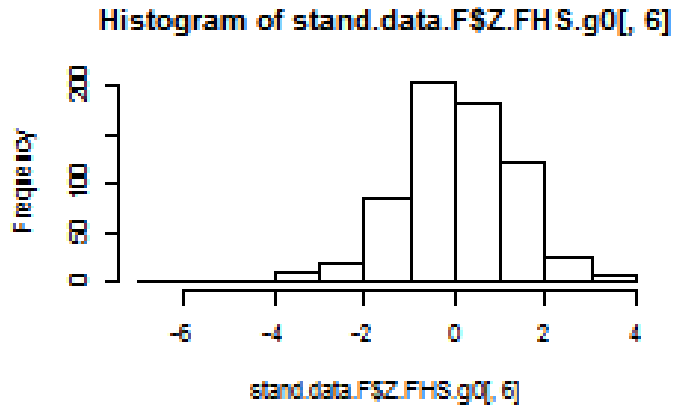


Histogram of stand.data.M\$Z.FHS.g3[, 6]

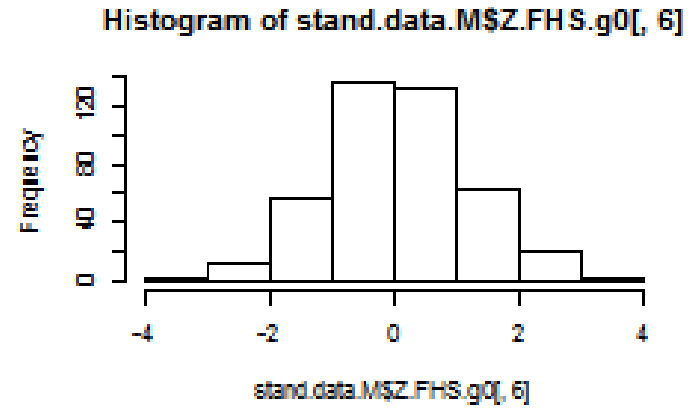


Supplement Figure S20-b: Externally Standardized Albumin

Females



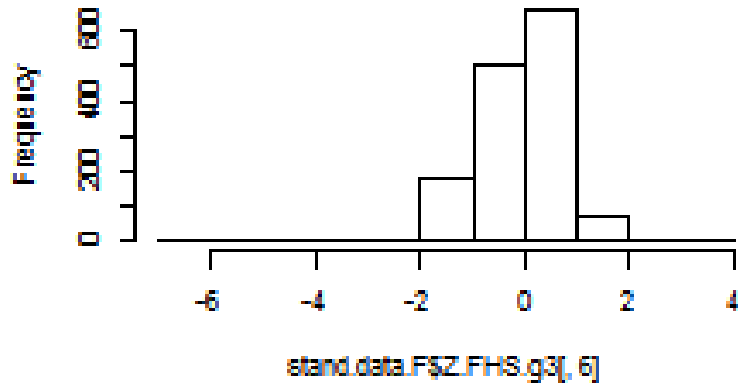
Males



Supplement Figure S20-c: Externally Standardized Abs. Monocyte Counts

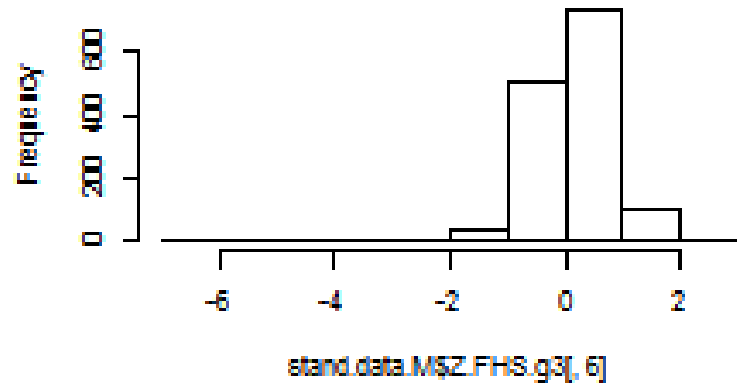
Females

Histogram of stand.data.F\$Z.FHS.g3[, 6]



Males

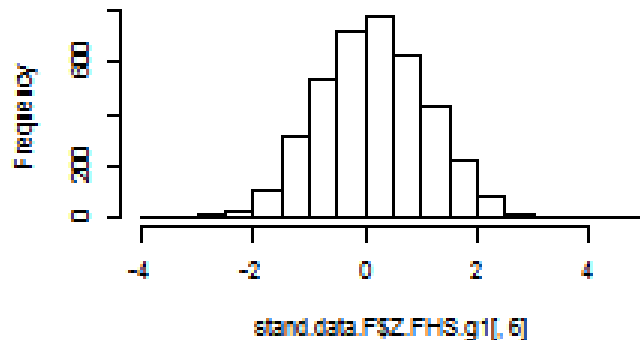
Histogram of stand.data.M\$Z.FHS.g3[, 6]



Supplement Figure S20-d: Externally Standardized NT-proBNP

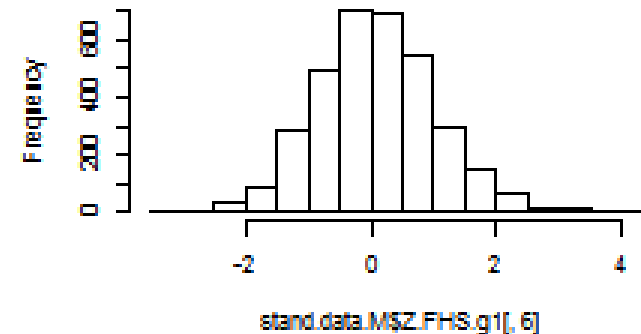
Females

Histogram of stand.data.F\$Z.FHS.g1[, 6]

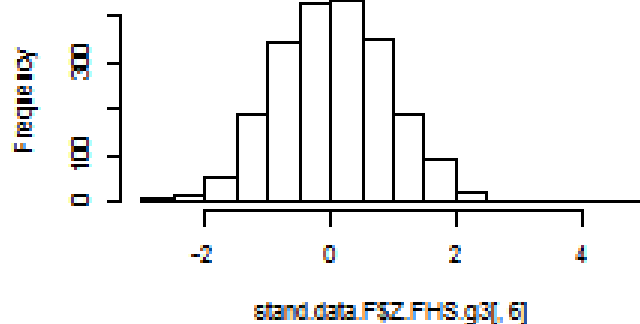


Males

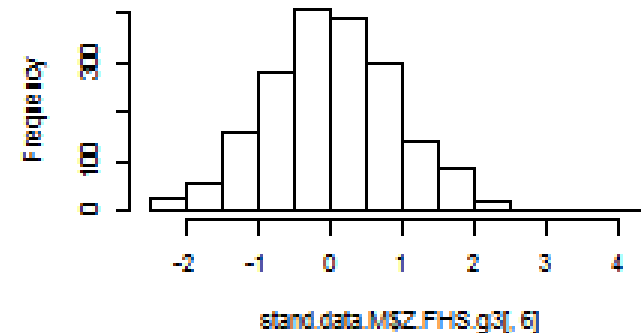
Histogram of stand.data.M\$Z.FHS.g1[, 6]



Histogram of stand.data.F\$Z.FHS.g3[, 6]

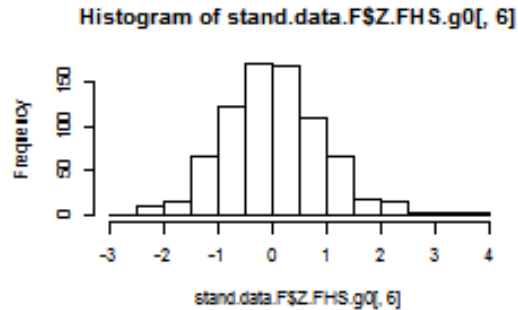


Histogram of stand.data.M\$Z.FHS.g3[, 6]

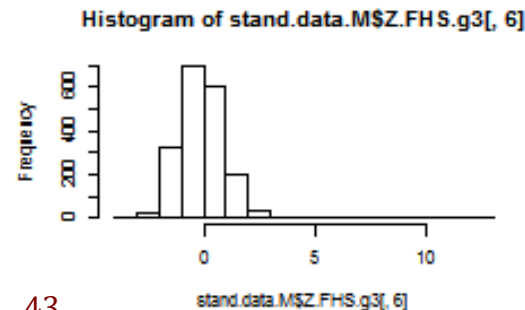
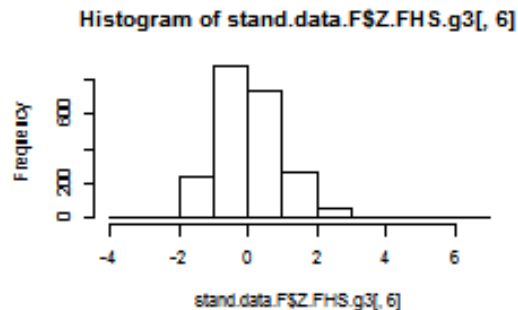
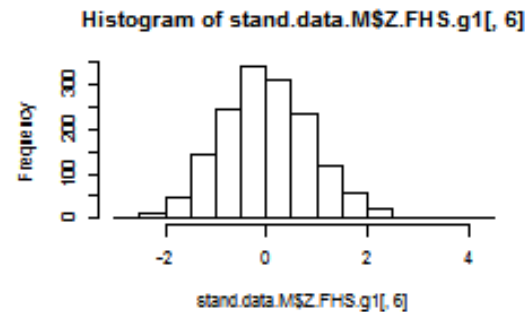
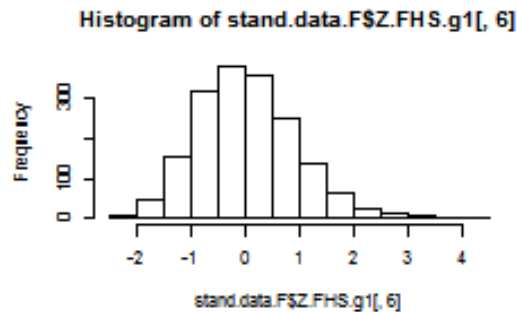
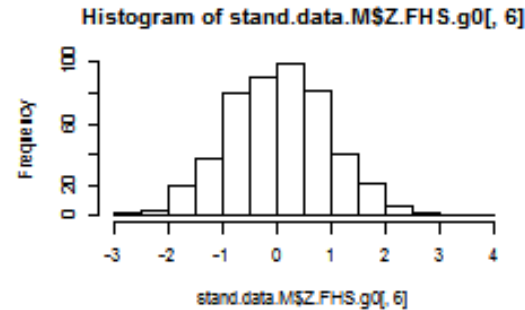


Supplement Figure S20-e: Externally Standardized Total Cholesterol

Females



Males

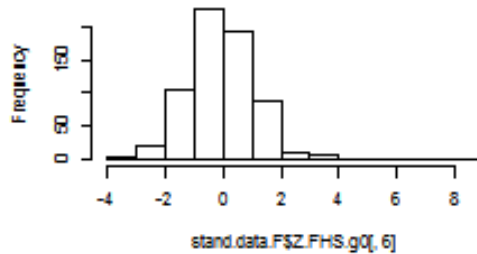


Supplement Figure S20-f: Externally Standardized Creatinine

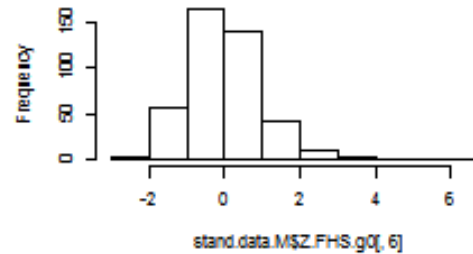
Females

Males

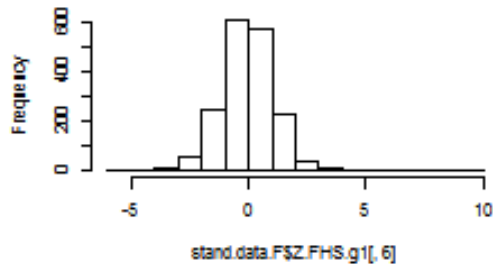
Histogram of stand.data.F\$Z.FHS.g0[, 6]



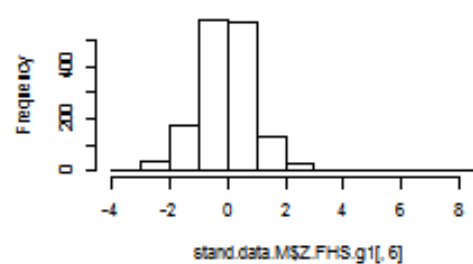
Histogram of stand.data.M\$Z.FHS.g0[, 6]



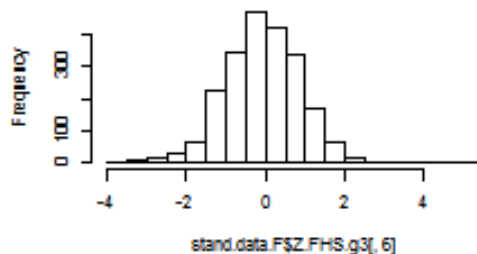
Histogram of stand.data.F\$Z.FHS.g1[, 6]



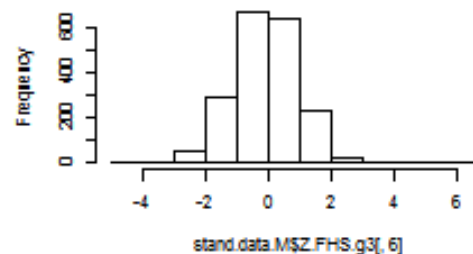
Histogram of stand.data.M\$Z.FHS.g1[, 6]



Histogram of stand.data.F\$Z.FHS.g3[, 6]



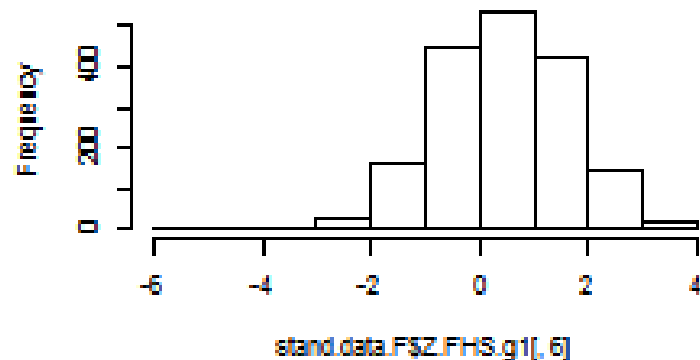
Histogram of stand.data.M\$Z.FHS.g3[, 6]



Supplement Figure S20-g: Externally Standardized CRP

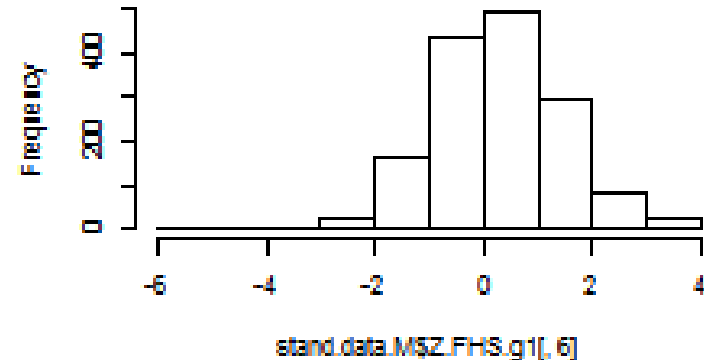
Females

Histogram of stand.data.F\$Z.FHS.g1[, 6]

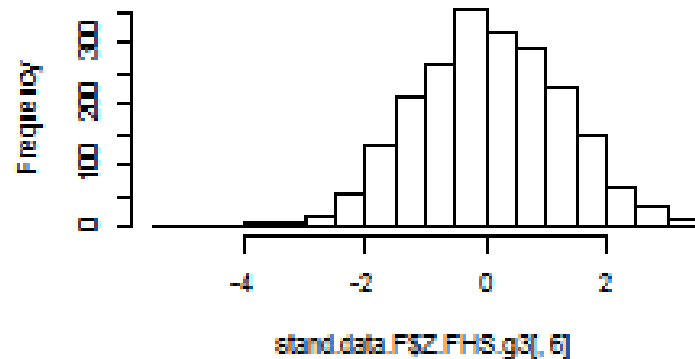


Males

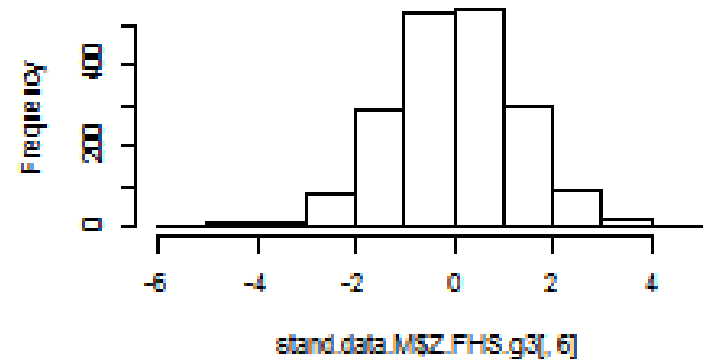
Histogram of stand.data.M\$Z.FHS.g1[, 6]



Histogram of stand.data.F\$Z.FHS.g3[, 6]



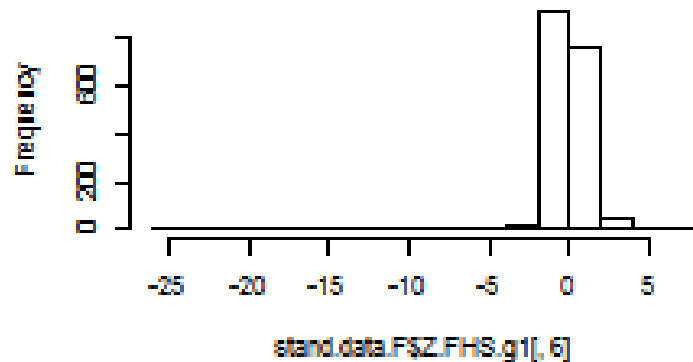
Histogram of stand.data.M\$Z.FHS.g3[, 6]



Supplement Figure S20-j: Externally Standardized Cystatin

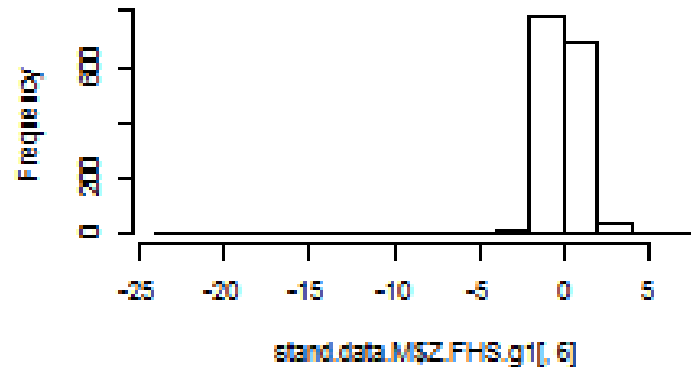
Females

Histogram of `stand.data.F$Z.FHS.g1[, 6]`

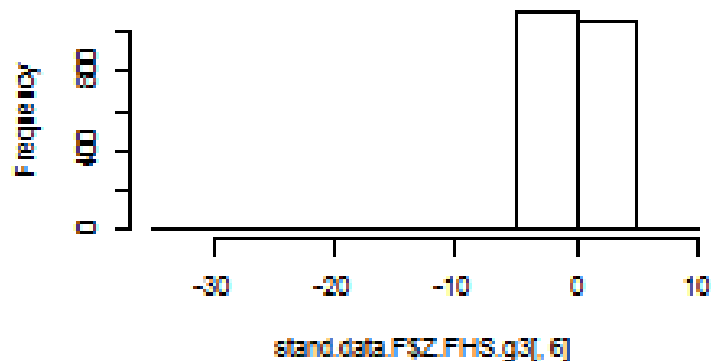


Males

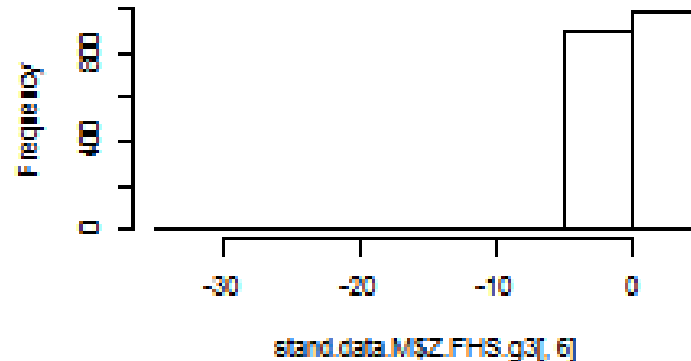
Histogram of `stand.data.M$Z.FHS.g1[, 6]`



Histogram of `stand.data.F$Z.FHS.g3[, 6]`



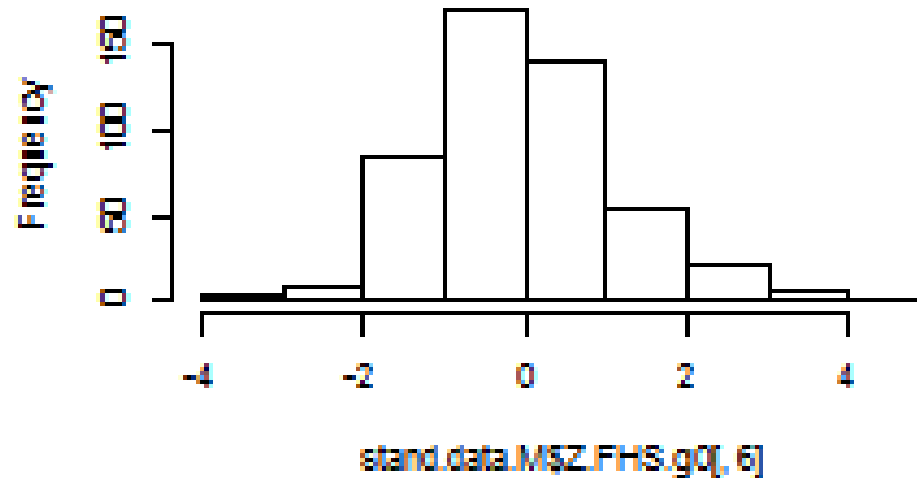
Histogram of `stand.data.M$Z.FHS.g3[, 6]`



Supplement Figure S20-k: Externally Standardized DHEA

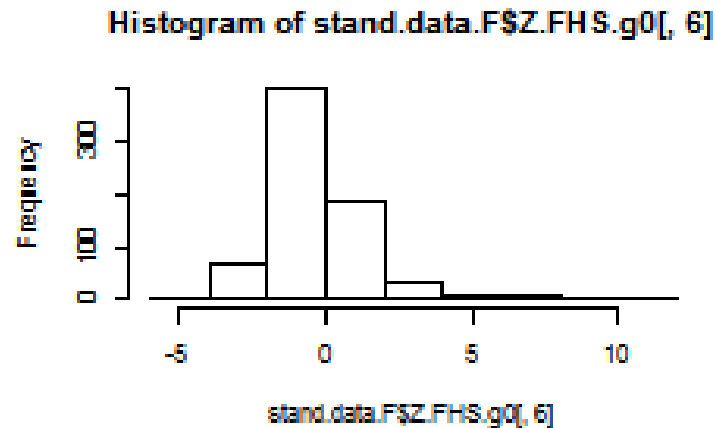
Males

Histogram of stand.data.M\$Z.FHS.g0[, 6]

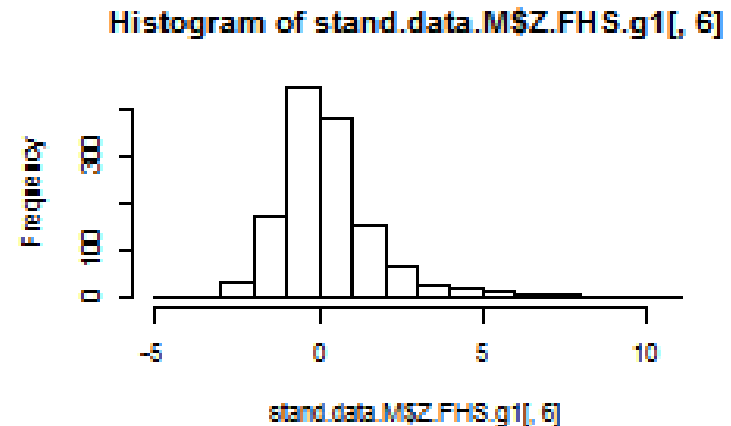
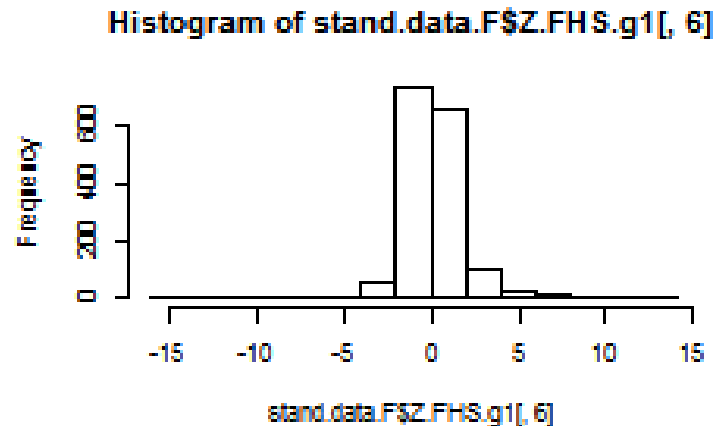
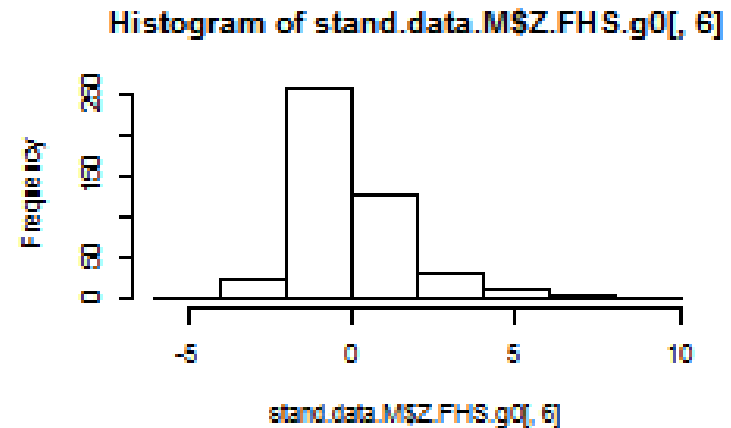


Supplement Figure S20-h: Externally Standardized HbA1C

Females



Males

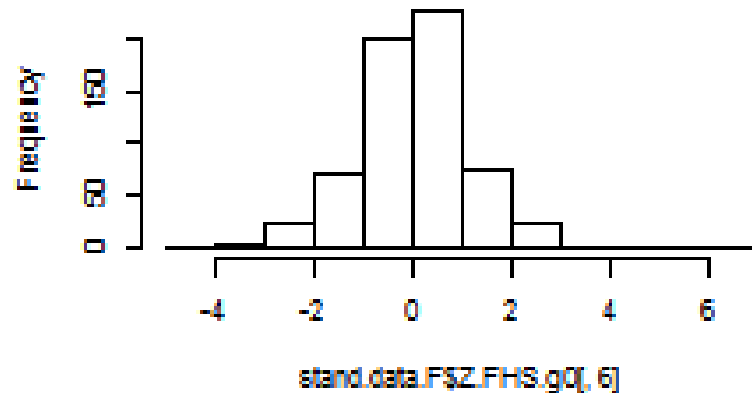


Supplement Figure S20-m: Externally Standardized HGB

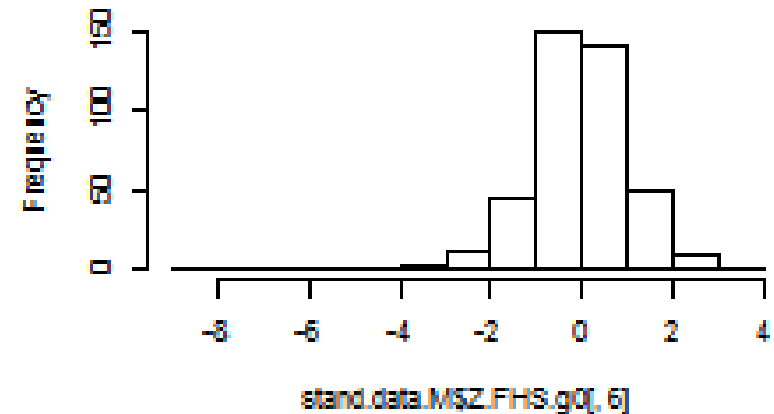
Females

Males

Histogram of stand.data.F\$Z.FHS.g0[, 6]



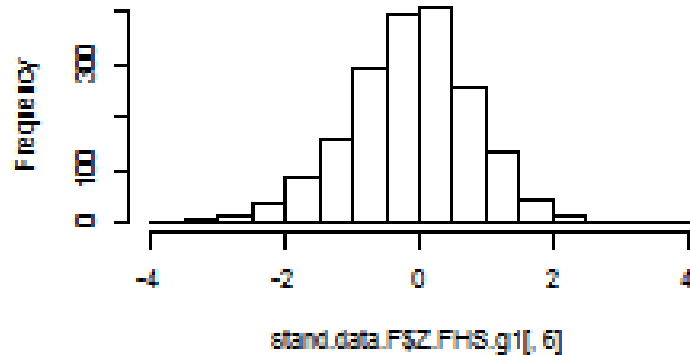
Histogram of stand.data.M\$Z.FHS.g0[, 6]



Supplement Figure S20-n: Externally Standardized IGF1

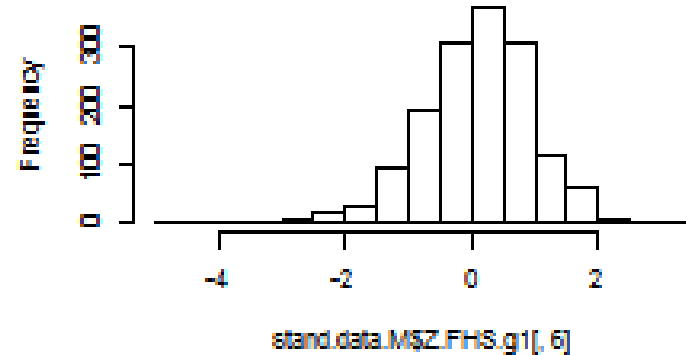
Females

Histogram of stand.data.F\$Z.FHS.g1[, 6]

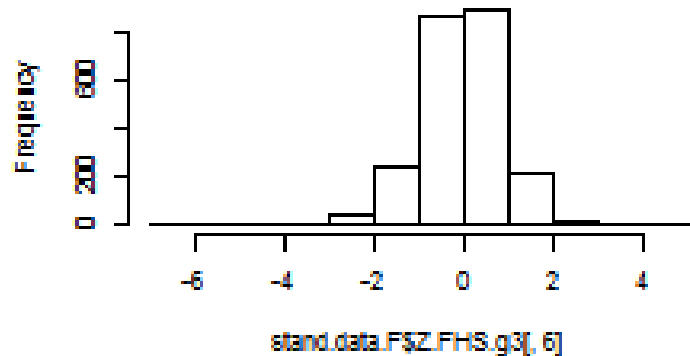


Males

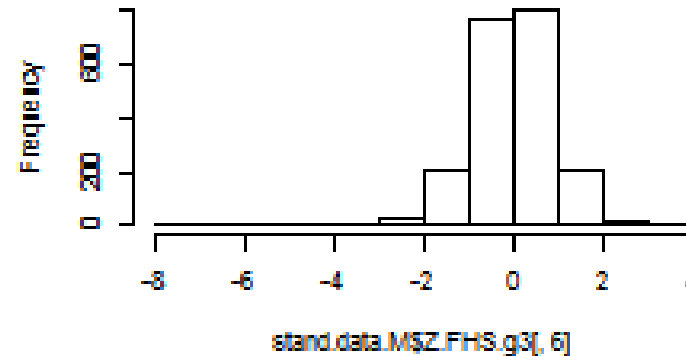
Histogram of stand.data.M\$Z.FHS.g1[, 6]



Histogram of stand.data.F\$Z.FHS.g3[, 6]



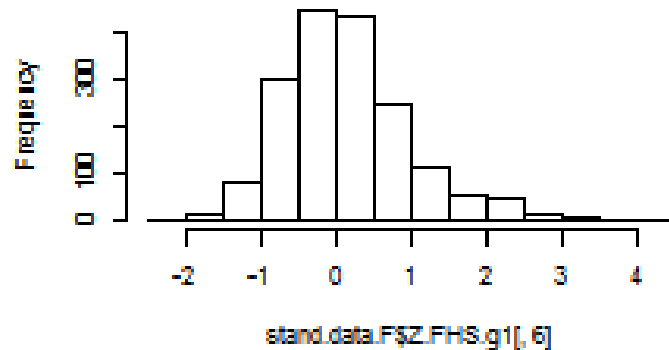
Histogram of stand.data.M\$Z.FHS.g3[, 6]



Supplement Figure S20-p: Externally Standardized IL6

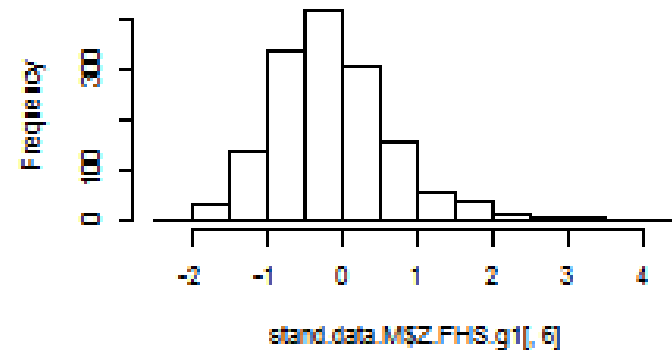
Females

Histogram of stand.data.F\$Z.FHS.g1[, 6]

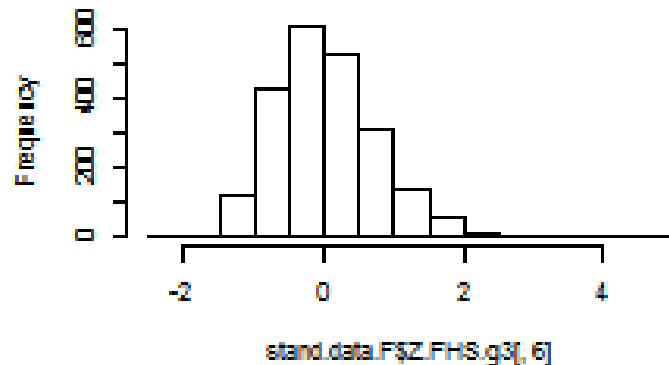


Males

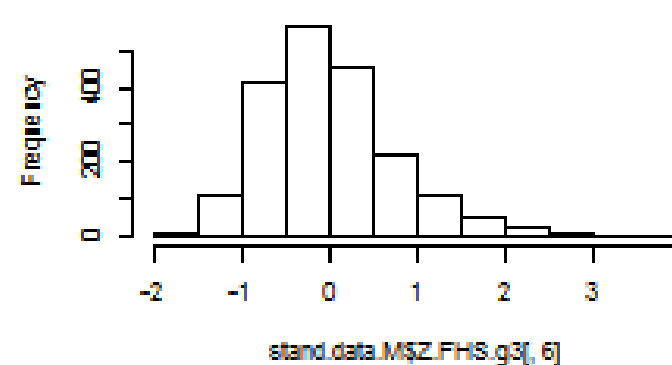
Histogram of stand.data.M\$Z.FHS.g1[, 6]



Histogram of stand.data.F\$Z.FHS.g3[, 6]

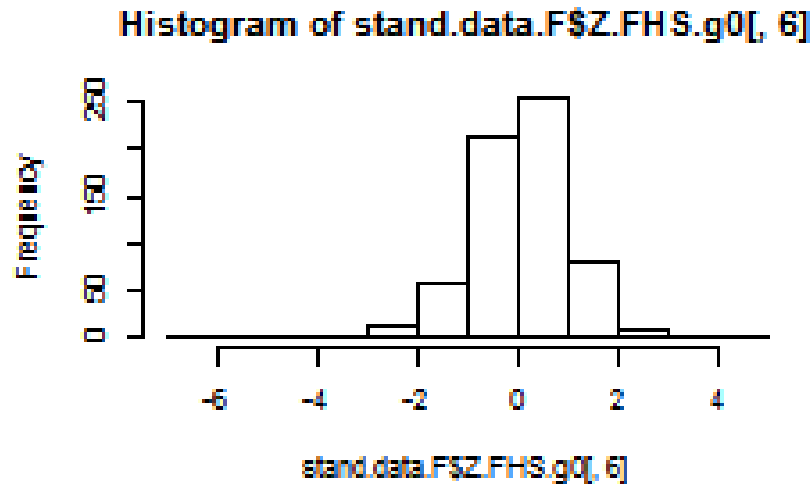


Histogram of stand.data.M\$Z.FHS.g3[, 6]

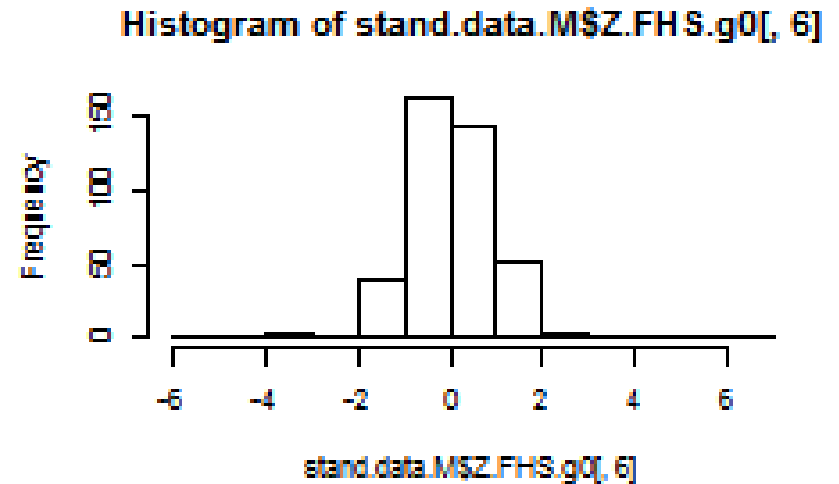


Supplement Figure S20-q: Externally Standardized MCV

Females



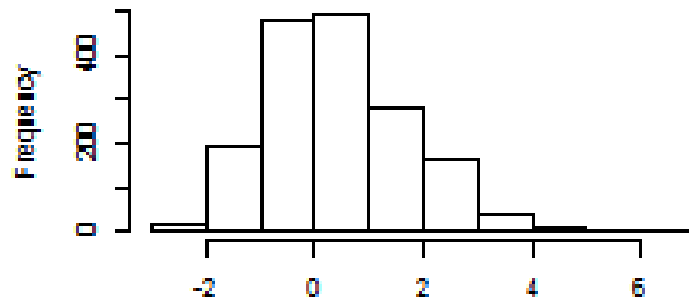
Males



Supplement Figure S20-r: Externally Standardized SHBG

Females

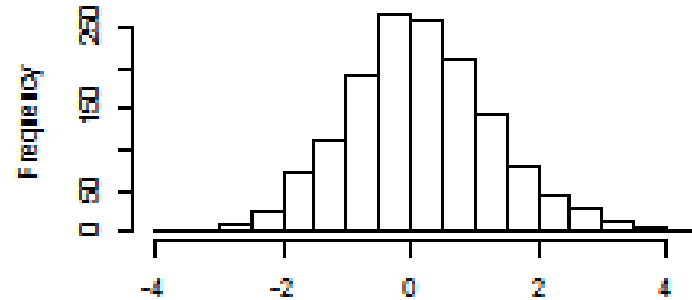
Histogram of stand.data.F\$Z.FHS.g1[, 6]



stand.data.F\$Z.FHS.g1[, 6]

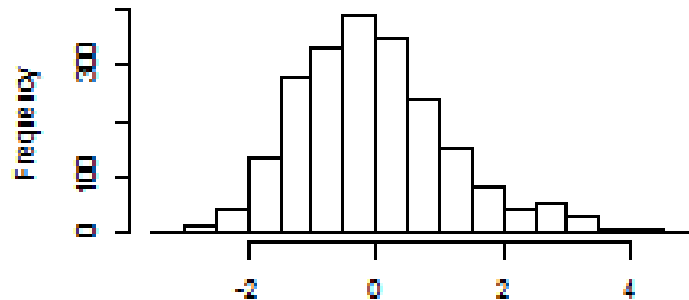
Males

Histogram of stand.data.M\$Z.FHS.g1[, 6]



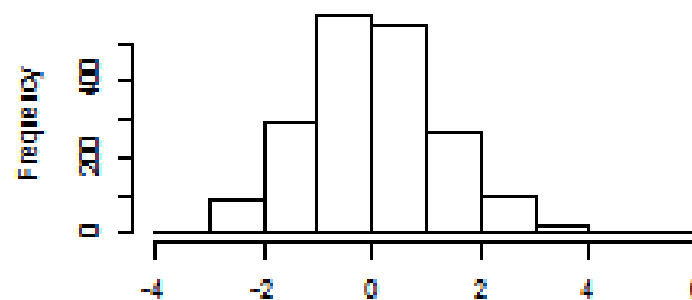
stand.data.M\$Z.FHS.g1[, 6]

Histogram of stand.data.F\$Z.FHS.g3[, 6]



stand.data.F\$Z.FHS.g3[, 6]

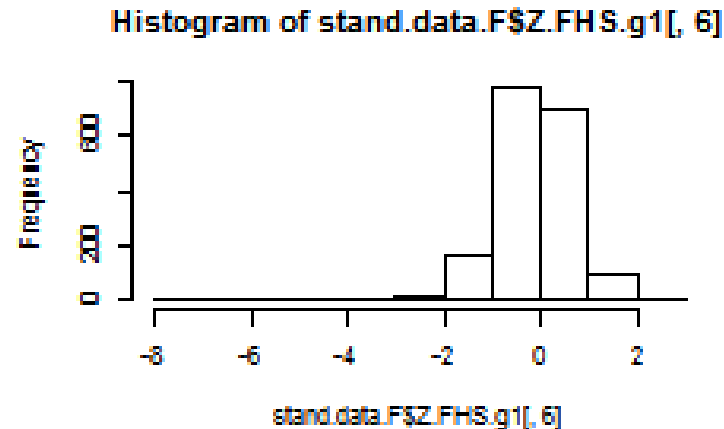
Histogram of stand.data.M\$Z.FHS.g3[, 6]



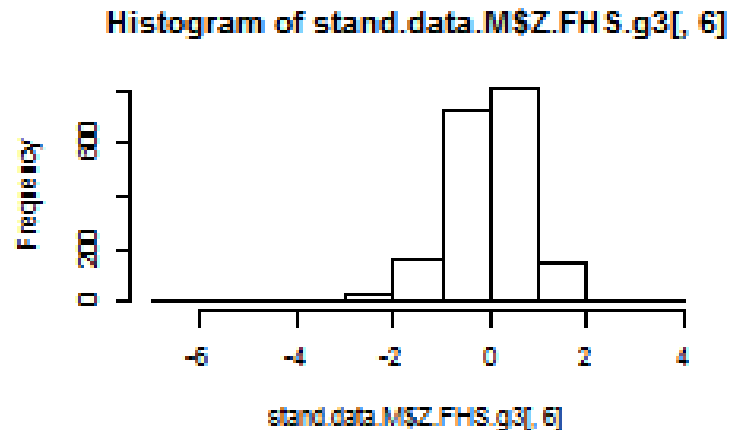
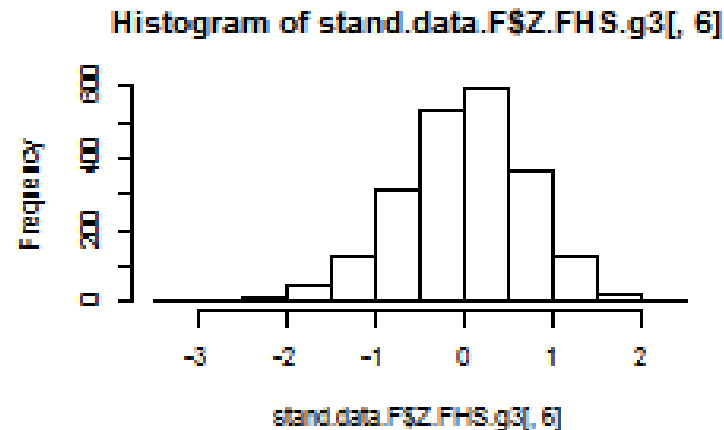
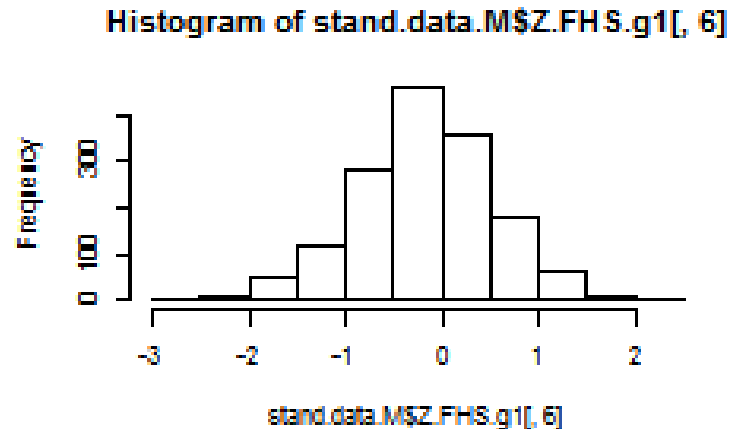
stand.data.M\$Z.FHS.g3[, 6]

Supplement Figure S20-s: Externally Standardized SRAGE

Females



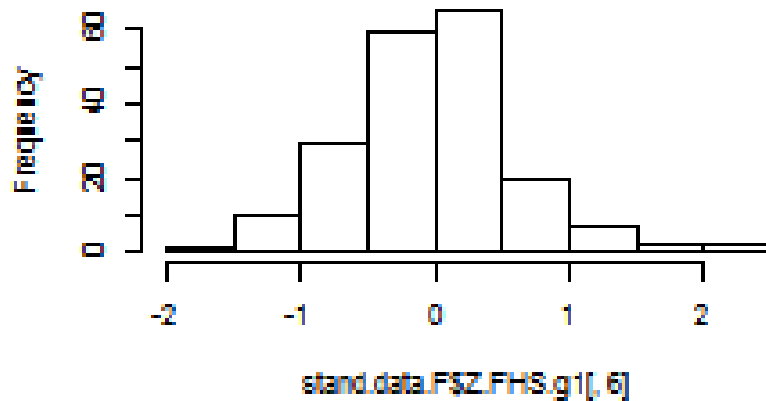
Males



Supplement Figure S20-v: Externally Standardized Transferrin

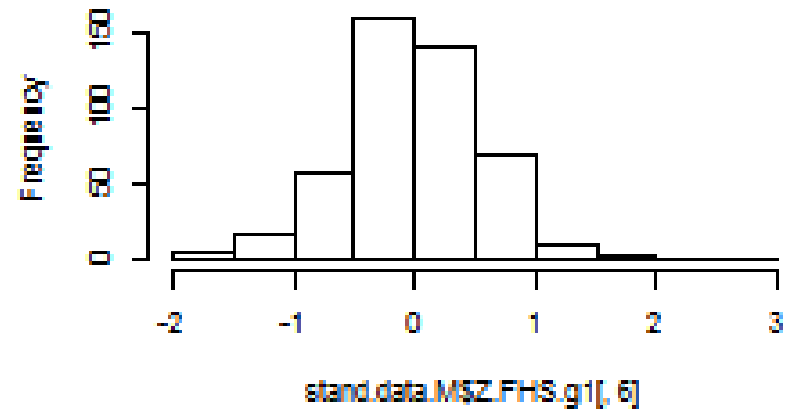
Females

Histogram of stand.data.F\$Z.FHS.g1[, 6]



Males

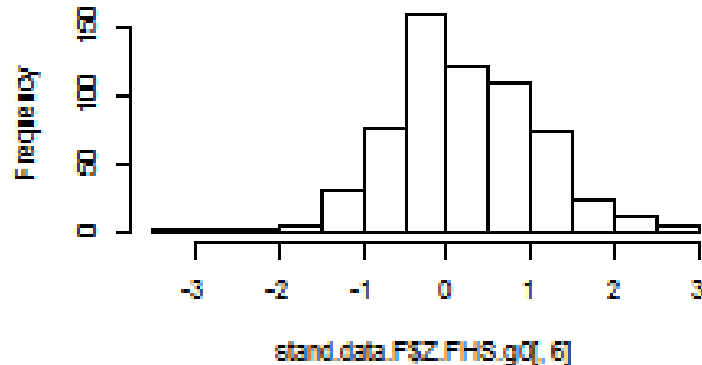
Histogram of stand.data.M\$Z.FHS.g1[, 6]



Supplement Figure S20-w: Externally Standardized WBC

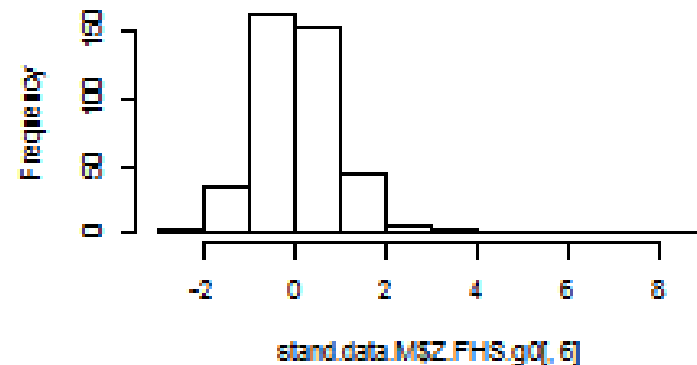
Females

Histogram of stand.data.F\$Z.FHS.g0[, 6]

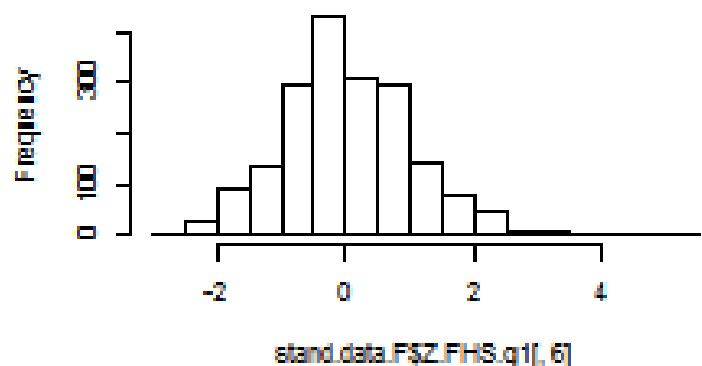


Males

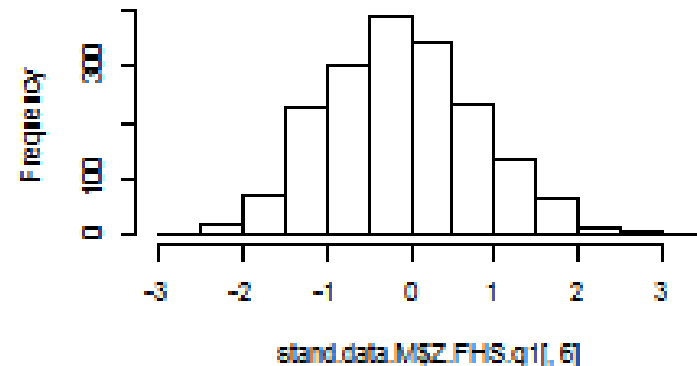
Histogram of stand.data.M\$Z.FHS.g0[, 6]



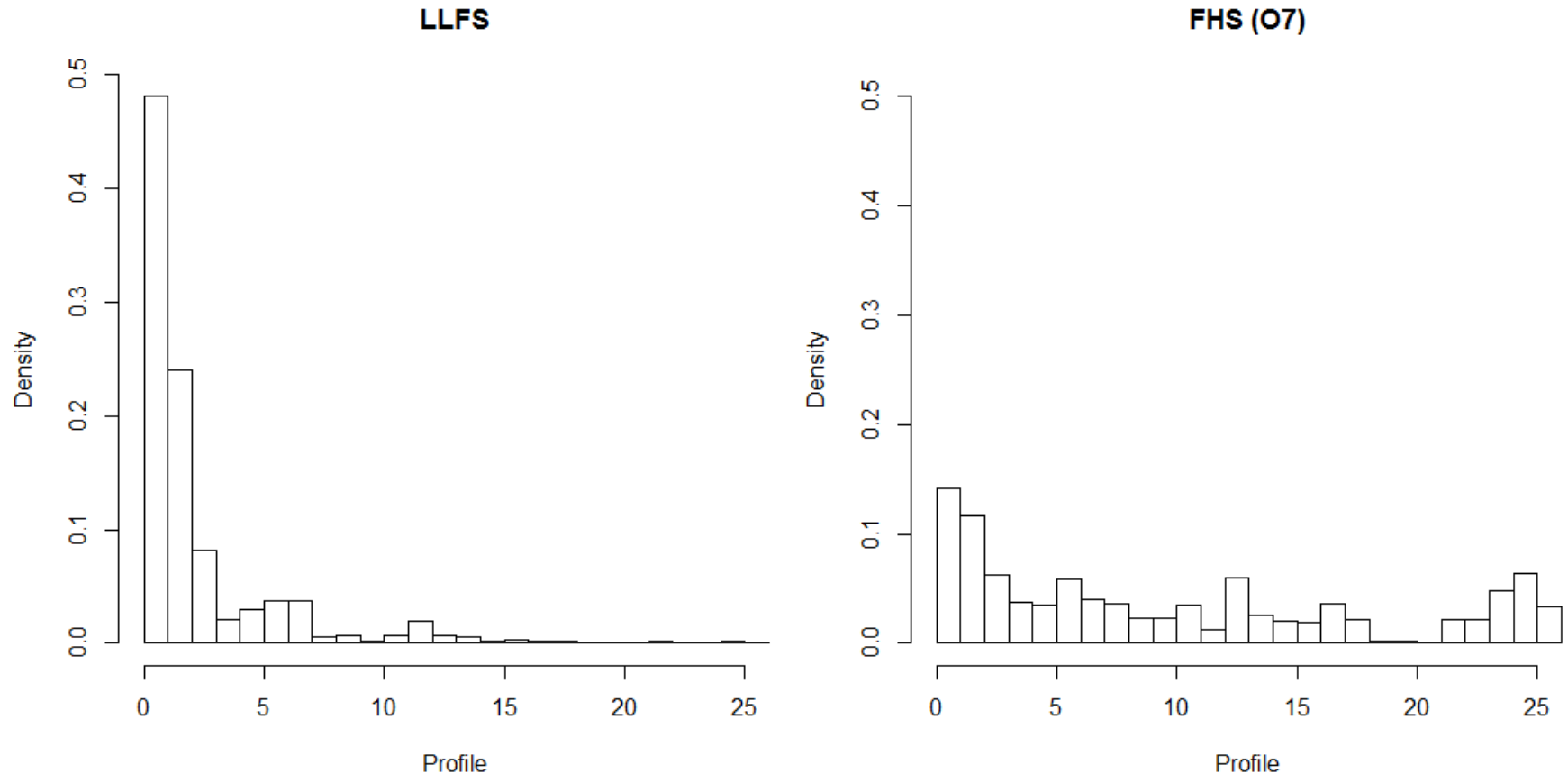
Histogram of stand.data.F\$Z.FHS.g1[, 6]



Histogram of stand.data.M\$Z.FHS.g1[, 6]



Supplement Figure S21: Distribution of Biomarker Signatures in LLFS and FHS



We see many more “anomalous patterns” in FHS

Figure S22-a Biomarker Signatures 1-4 in FHS

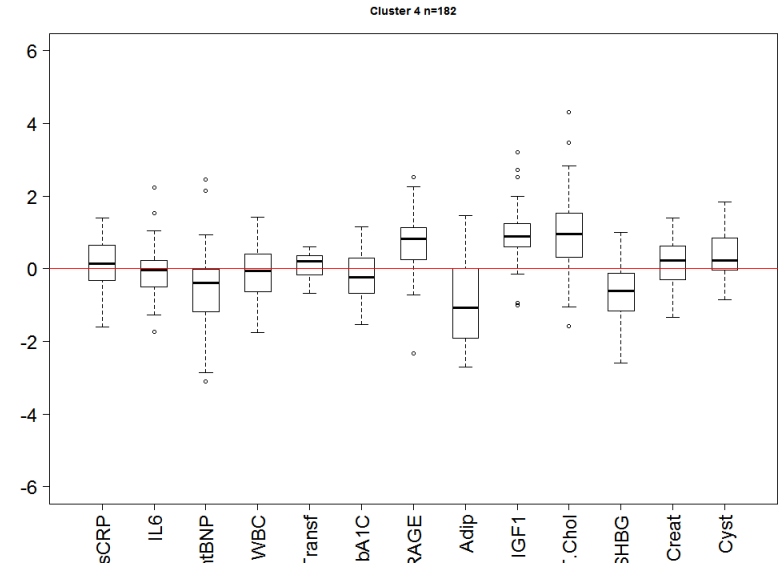
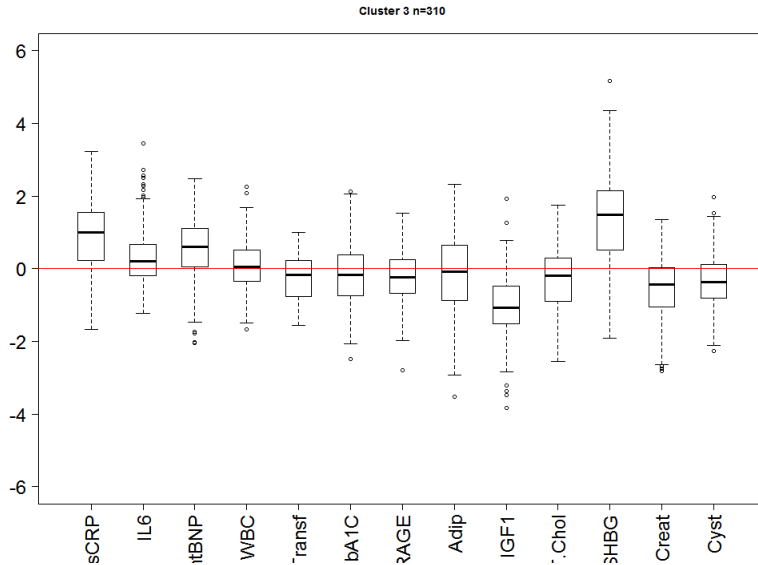
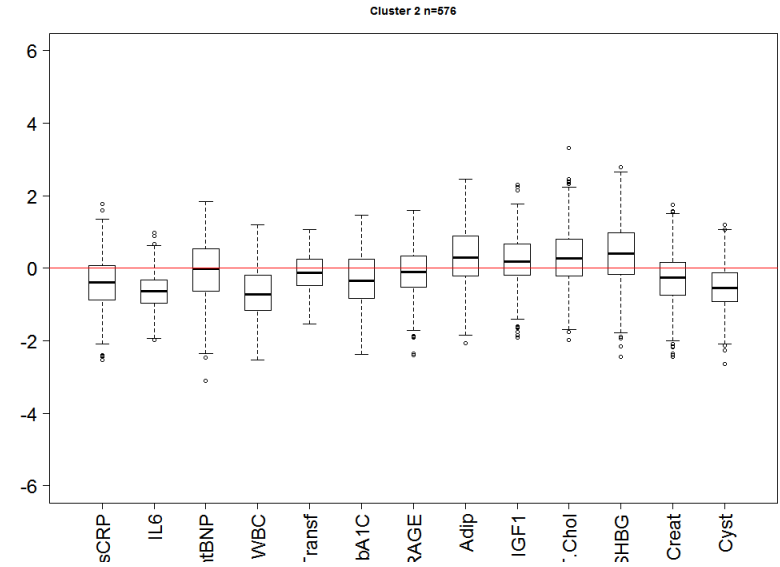
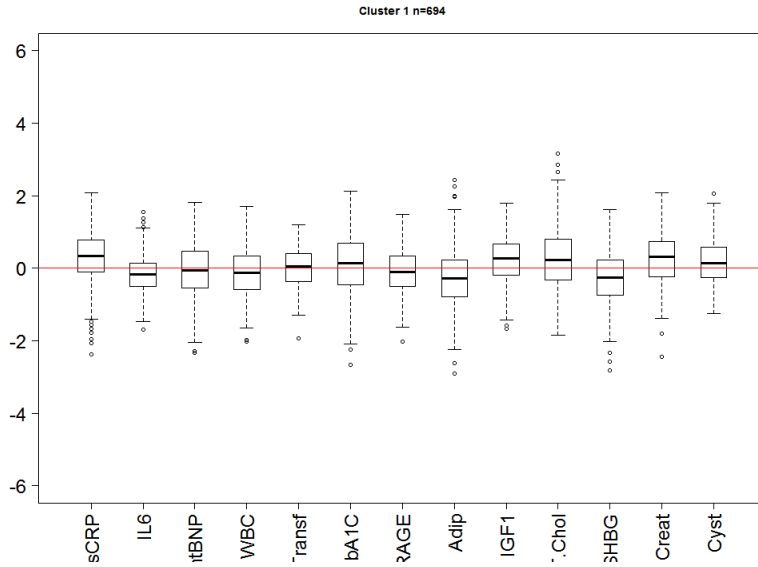


Figure S22-b Biomarker Signatures 5-8 in FHS

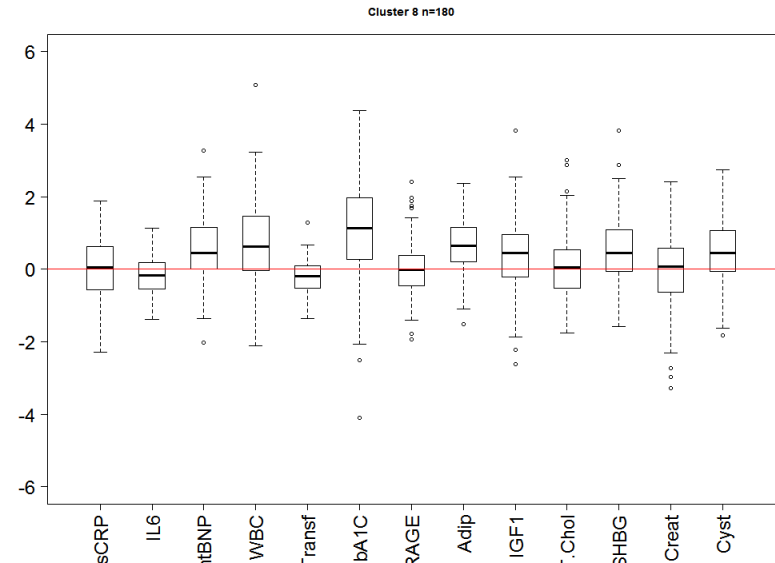
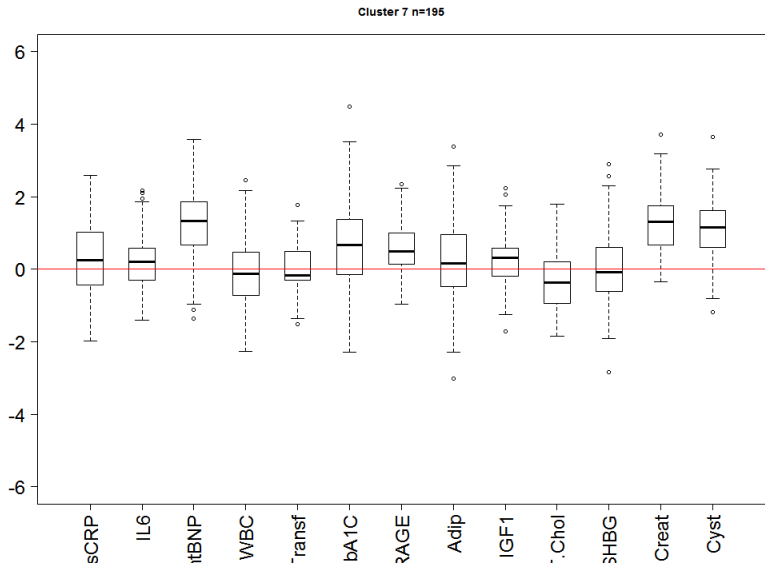
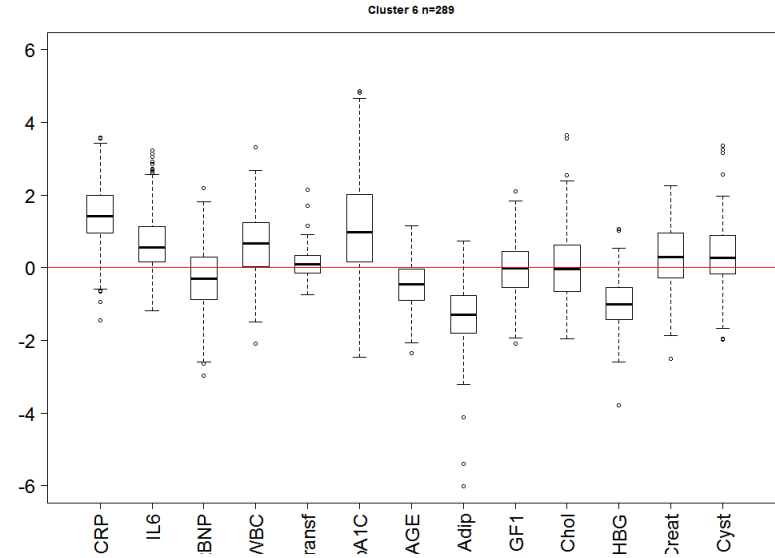
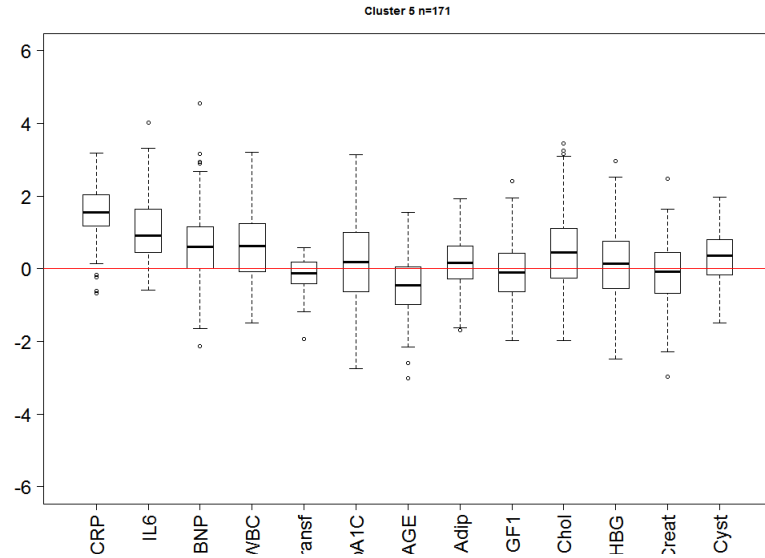


Figure S22-c Biomarker Signatures 9-12 in FHS

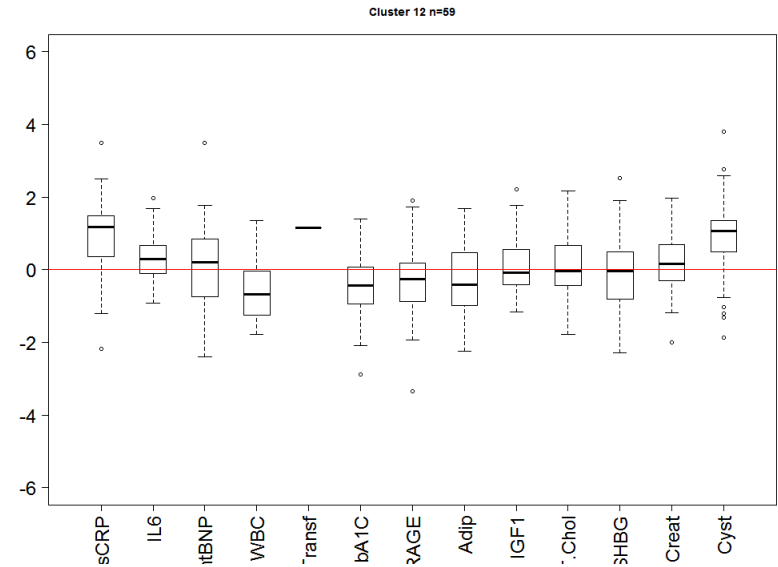
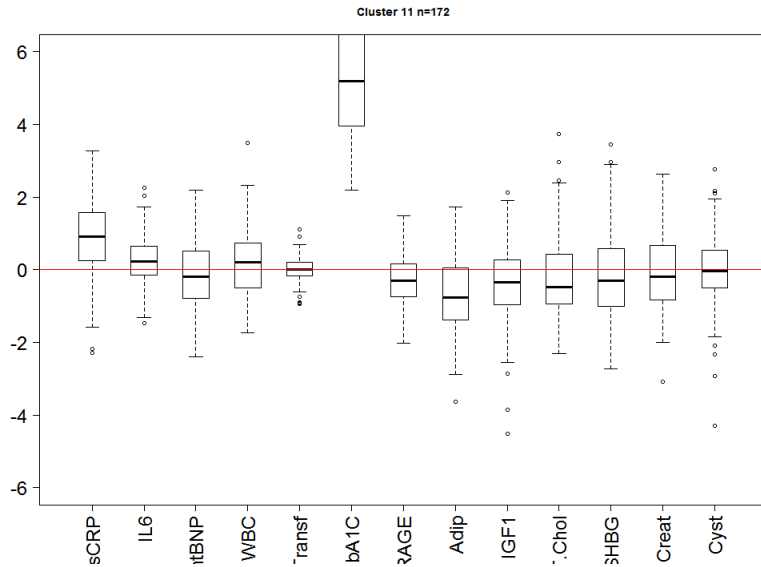
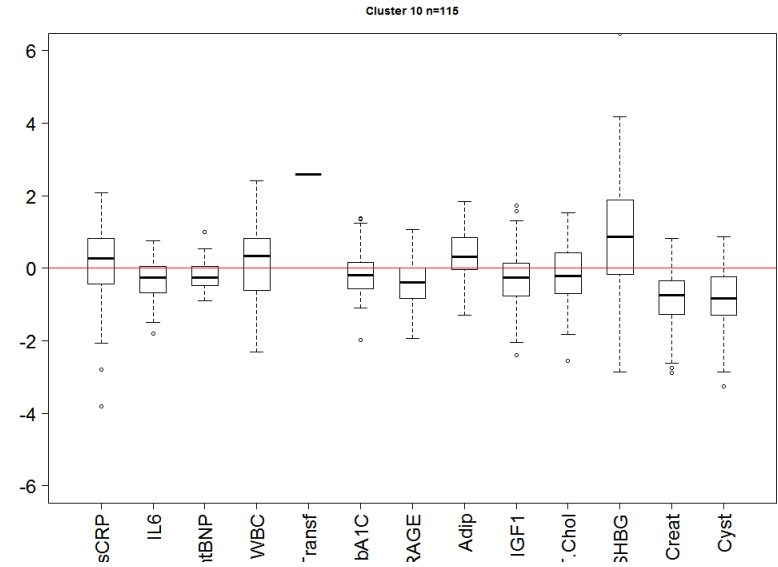
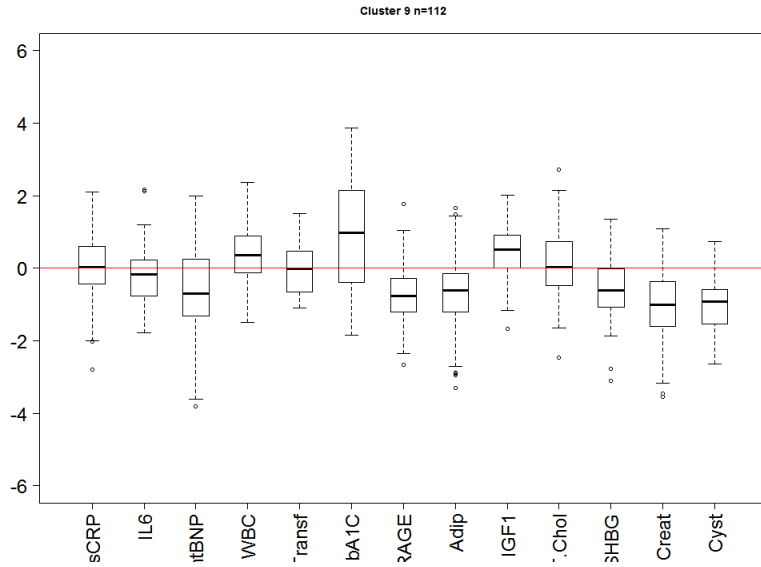


Figure S22-d Biomarker Signatures 13-16 in FHS

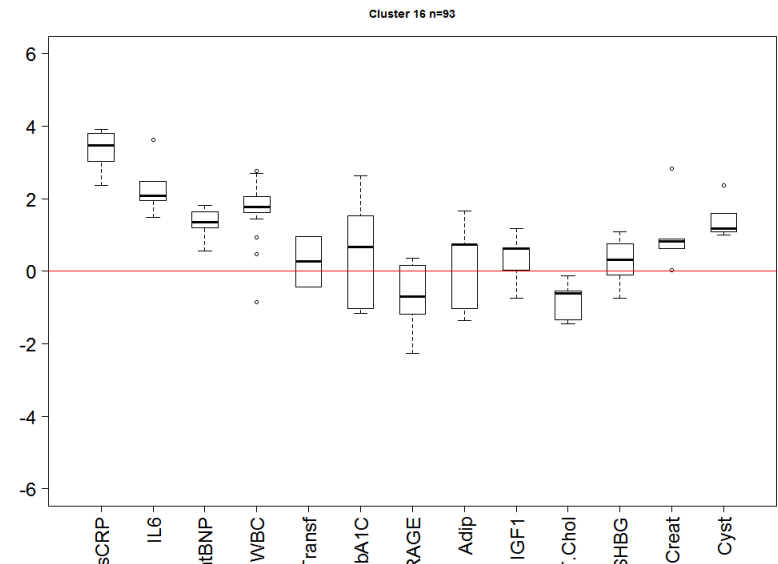
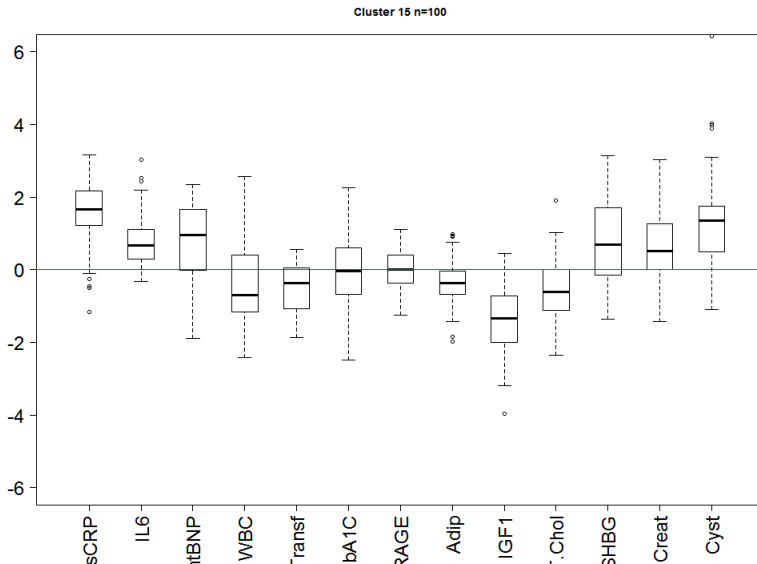
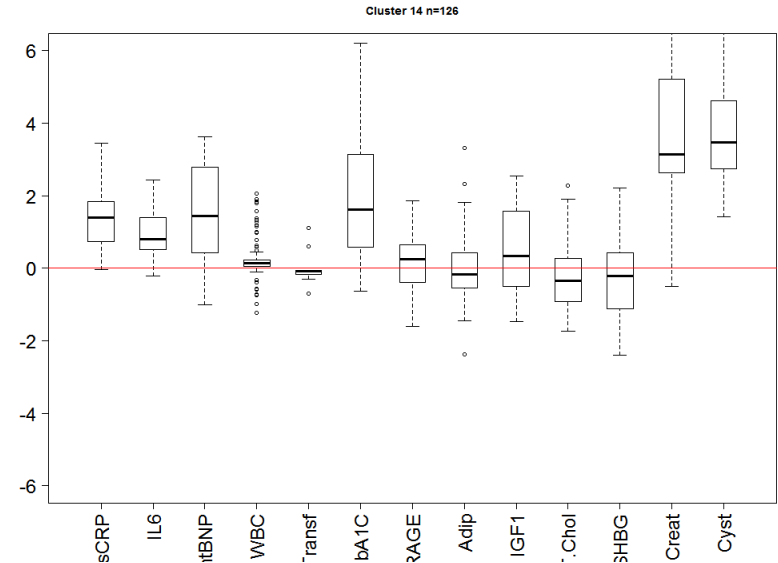
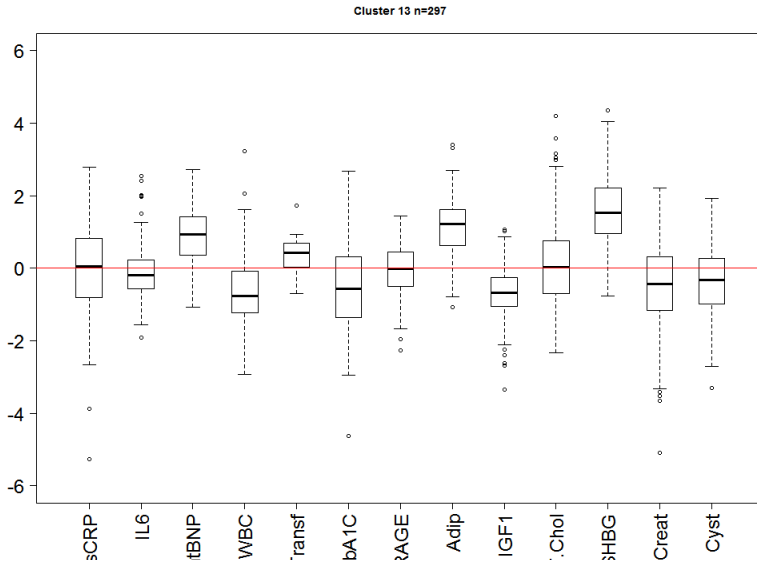


Figure S22-e Biomarker Signatures 17-20 in FHS

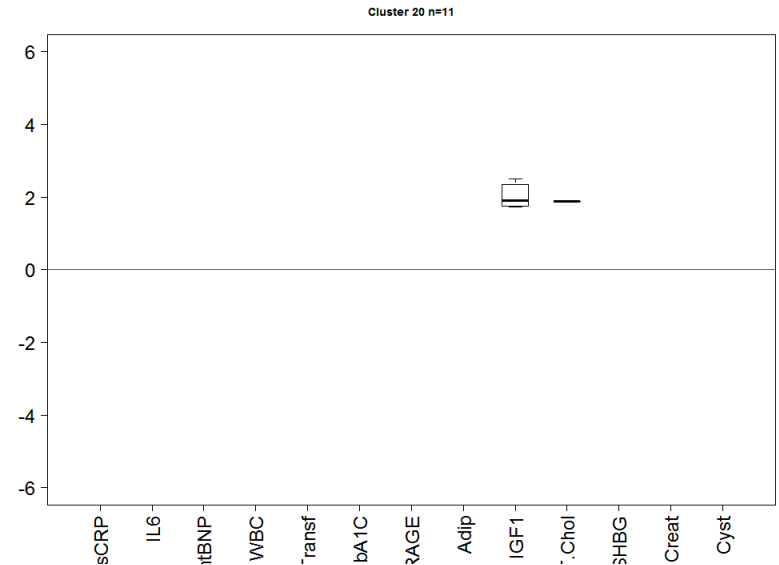
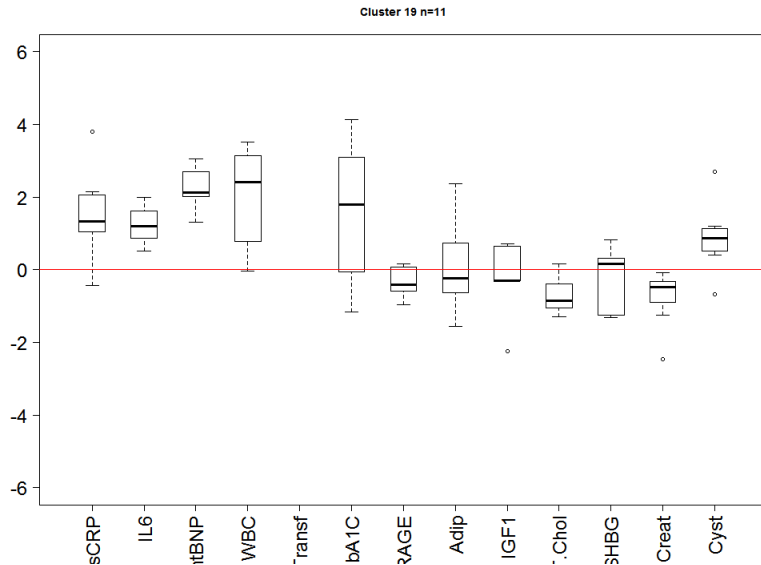
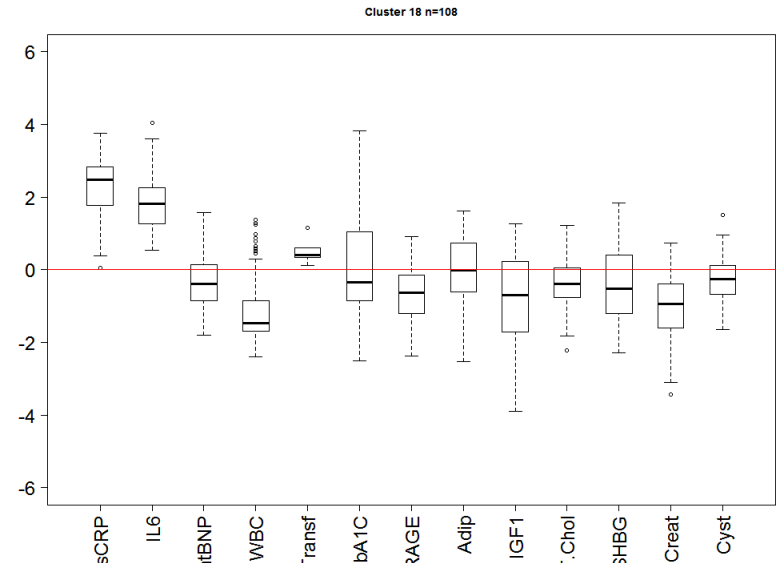
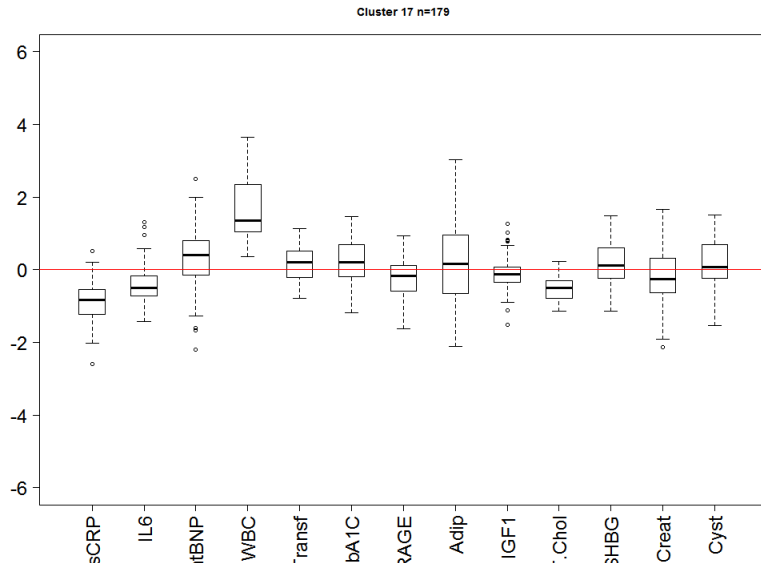


Figure S22-f Biomarker Signatures 21-24 in FHS

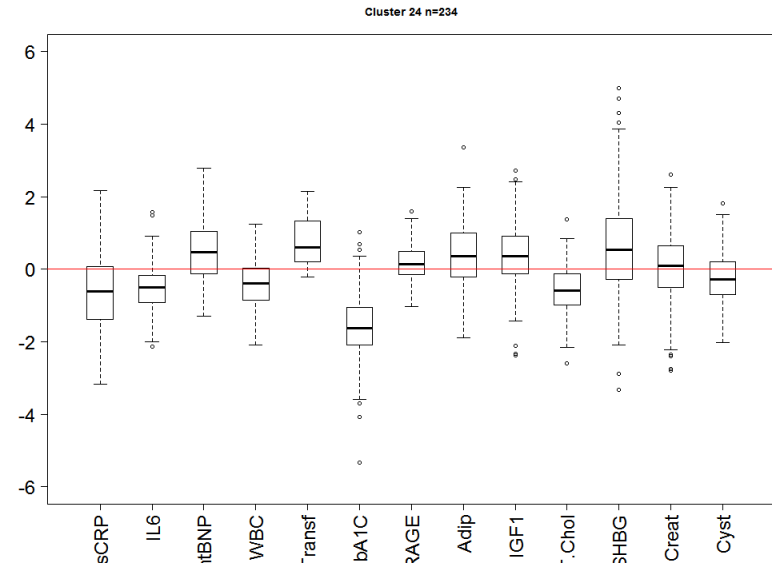
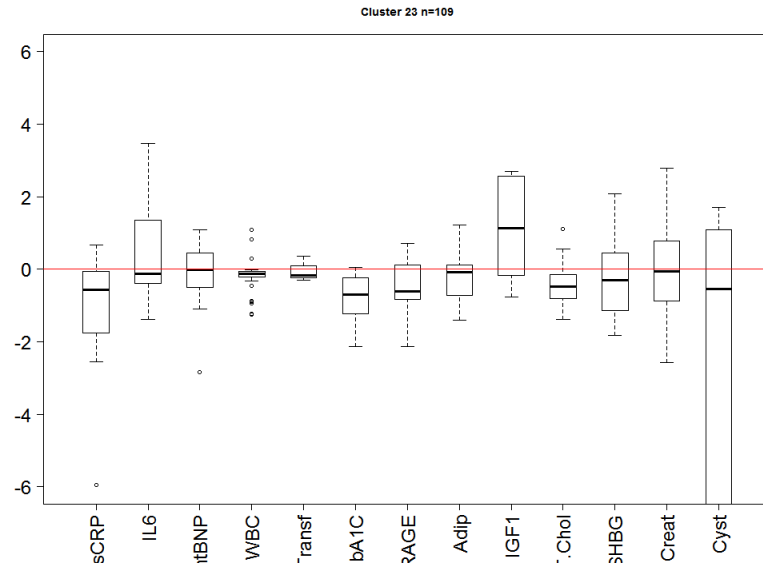
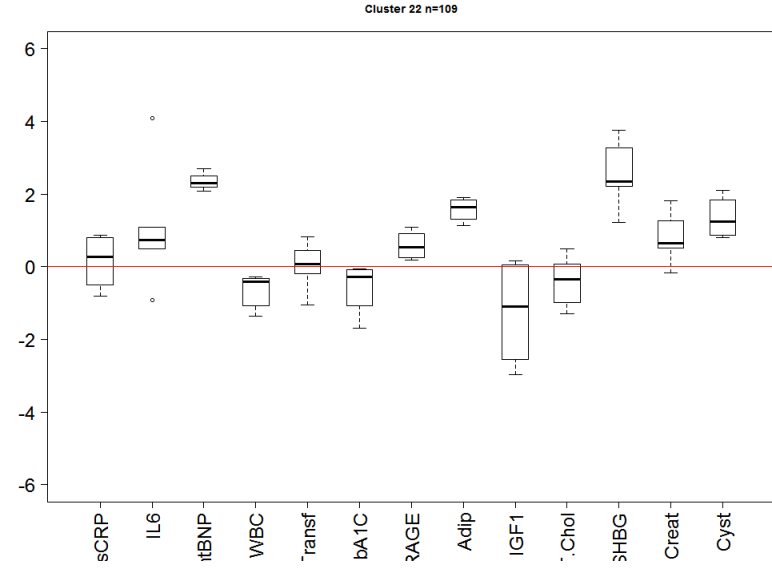
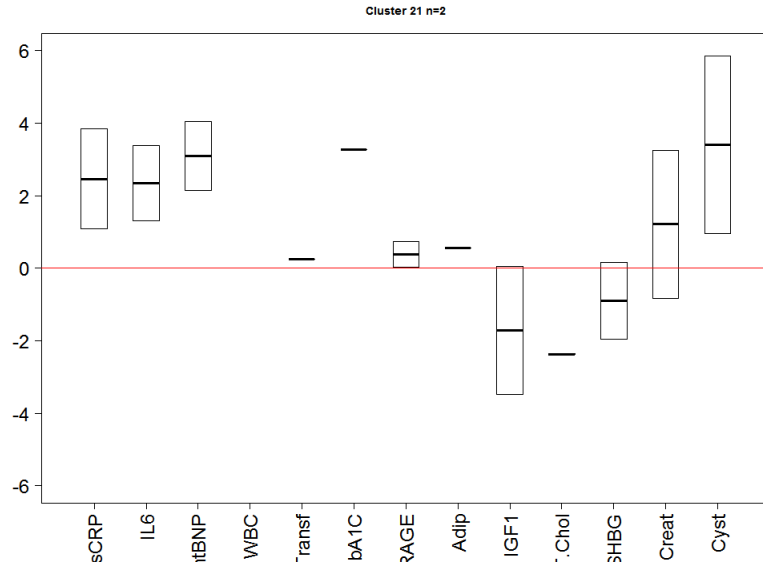


Figure S22-g Biomarker Signatures 25, 26 in FHS

